MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

Vol. XLIII JULY-AUGUST, 1951 No. 4

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[MYCOLOGIA for May–June (43: 249–387) was issued July 3, 1951.]

PUBLISHED BIMONTHLY FOR
THE NEW YORK BOTANICAL GARDEN
AT PRINCE AND LEMON STS., LANCASTER, PA.

Entered as second-class matter April 30, 1925, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

MYCOLOGIA

Published by

THE NEW YORK BOTANICAL GARDEN

IN COLLABORATION WITH THE

MYCOLOGICAL SOCIETY OF AMERICA

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THE EFFECT OF ADDED B-VITAMINS ON THE GROWTH AND ESTER PRODUCTION OF HANSENULA ANOMALA (HANSEN) SYDOW [1]

CALVIN C. KUEHNER

(WITH 3 FIGURES)

In his review of the work done in the field of "mold" biochemistry, Birkinshaw (**1937**) has emphasized the fact that although the taxonomic work relating to the lower fungi encompasses two centuries of descriptive botany the study of the biochemistry of these organisms is of comparatively recent origin. Yeast studies in particular have been numerous but most of them have been confined to varieties of *Saccharomyces cerevisiae* Hansen, the common yeast of industry. There exists a field, practically untouched, in the study of the physiology of the "wild" or "weed" yeasts of which group *Hansenula anomala* (Hansen) Sydow is a member. Gray (**1949**) has demonstrated the production of ethyl acetate by this organism, furnishing one example of the importance of further study of genera of yeasts other than *Saccharomyces*. An increased knowledge of the physiology of these yeasts may not only provide commercially valuable methods of synthesis of desirable compounds, but, from a taxonomic viewpoint, it might make possible the classification of yeasts on some more satisfactory basis than now exists.

[1] Paper No. 527 from the Department of Botany, The Ohio State University. Based on a thesis submitted in partial fulfillment of the requirements for the degree Master of Science. The writer is indebted to Dr. W. D. Gray for his advice during the course of the work.

[MYCOLOGIA for May–June (**43**: 249–387) was issued July 3, 1951]

These studies were undertaken in order to determine the vitamin deficiencies, if any, and the effects of certain vitamins upon the growth of *Hansenula anomala*. Since various B-vitamins have been shown to play an important role in certain enzyme systems involved in glycolysis the question naturally arose as to whether added amounts of these vitamins would have an accelerating effect upon the ester production of this yeast. It soon became evident that there was an interaction between added vitamins and trace elements. This report is concerned primarily with vitamin effects and critical trace element studies must be made to further clarify this problem.

MATERIALS AND METHODS

The strain of *H. anomala* used in these studies was obtained originally in 1939 from the Department of Agricultural Bacteriology, University of Wisconsin, by Joseph E. Seagram and Sons, Inc., and is listed as No. 20 in their stock culture collection.

Stock cultures were maintained on Difco Yeast Extract agar slants and were transferred every 72 hr. during the course of the experiments. Inoculum was prepared by removing a small amount of cells from a 72-hour-old culture with a standard loop made from nichrome wire. The diameter of the loop was 3.0 mm. and it held approximately 0.06 ml. The cells so removed were then placed in 100 ml. of the sterile basal medium containing no added vitamins or trace elements. This culture was incubated without agitation for 24 hr. at 28° C before use as inoculum. In the growth studies a standard loopful of inoculum was introduced into each culture bottle while in the yield studies 1.0 ml. of the inoculum was added by means of a sterile 1.0 ml. graduated pipette.

The basal medium for growth studies was essentially that used by Burkholder (**1943**) in his studies of the vitamin requirements of thirty-eight yeasts. It contained the following:

Dextrose	20.0 grams
Asparagine (recrystallized)	2.0 grams
KH_2PO_4	1.5 grams
$MgSO_4 \cdot 7H_2O$	0.5 gram
$CaCl_2 \cdot 2H_2O$	0.33 gram
$(NH_4)_2SO_4$	2.0 grams
KI	0.1 milligram
Double distilled water to make	1.0 liter

Vitamins were added singly or in combination in micrograms per liter as follows: riboflavin, 100; pyridoxin hydrochloride, 200; thiamin hydrochloride, 200; niacin, 200; biotin methyl ester, 0.2; calcium pantothenate, 200; inositol, 1000; choline, 100; *p*-aminobenzoic acid, 100. Vitamin stock solutions were prepared as outlined in Koch and Hanke (**1948**) and were stored under toluene in the refrigerator.

Trace elements were added in milligrams per liter as follows: manganese (as manganous sulfate) 0.01; zinc (as zinc chloride) 0.07; boron (as boric acid) 0.01; copper (as cupric chloride) 0.01; iron (as ferrous sulfate) 0.05; molybdenum (as molybdenum trioxide) 0.01.

Hydrogen-ion concentration was measured with a Beckmann line-operated glass electrode. The pH of the medium was adjusted in all cases with 0.1 *N* sodium hydroxide or 0.1 *N* hydrochloric acid to read 5.0 after autoclaving.

All reagents used were C.P. grade. The dextrose was further purified by heating a water solution containing Norit A for 30–40 min. at a temperature just below the boiling point of the solution. Norit A was added at the rate of 1 gram per each 50 ml. of solution to be purified. The Norit was filtered out before the dextrose solution was added to the culture medium. Sugar determinations made by the method of Stiles, Peterson, and Fred (**1926**) indicate that not over 0.5 per cent dextrose is lost in the purification process. Asparagine was twice recrystallized from water, dried in a vacuum oven, and placed in a calcium chloride desiccator for at least 12 hours before use. Pyrex glassware was used in all cases except the growth studies, and all glassware was scrupulously cleaned by the usual laboratory techniques and rinsed at least ten times in tap water and five times in double distilled water. Double distilled water was used in the preparation of all media and stock solutions.

For the growth studies twenty-two kinds of media were prepared as follows: (1) basal medium; (2) basal medium plus all vitamins; (3) basal medium plus all but one vitamin (nine types of media); (4) basal medium plus one vitamin (nine types of media); (5) basal medium plus all vitamins plus 10 ml. of vitamin-free casein hydrolysate per liter; (6) basal medium plus all vita-

mins but biotin. Avidin was added to the last medium to clear it of any contaminating biotin. Trace elements were added to all of the above media.

The method of Gaines and Stahly (**1943**) was used to obtain the avidin (medium 6, above). Egg white was removed from an egg with a sterile hypodermic syringe after sterilization of the shell and inner membrane with 1.0 per cent $HgCl_2$ which was rinsed off with 95 per cent ethyl alcohol. The egg white was made to a final concentration of 30 per cent with sterile physiological saline. This solution was added to the medium at the rate of 0.5 ml. per each 10 ml. of medium. To prevent heat coagulation of the protein and subsequent inactivation of the avidin egg white was added to the medium after the medium had been autoclaved.

The six kinds of media used in the ester yield studies were: (1) basal medium plus all vitamins but no trace elements; (2) basal medium plus all vitamins and all trace elements; (3) basal medium plus trace elements; (4) basal medium only; (5) basal medium plus all vitamins and three times the usual amount of trace elements; (6) basal medium plus three times the usual amount of trace elements.

Media were sterilized by autoclaving at 15 lb. gauge pressure for 20 minutes.

The growth studies were conducted by using 10 ml. portions of medium in two ounce French Square bottles. These bottles permitted vigorous shaking with glass beads to break up the surface film and to suspend the yeast cells more uniformly before the amount of growth was determined. The bottles also afforded a uniform surface area whereas culture tubes might become slanted and thus increase the surface area and the degree of aeration. Since yield studies required a larger fermentation than growth studies, 250 ml. Erlenmeyer flasks containing 100 ml. portions of medium were used.

After incubation at 28° C for various lengths of time the turbidity of the cultures was measured with a Klett-Summerson Photoelectric colorimeter using a No. 42 (blue) filter. The readings were taken as an indication of the extent of growth of the yeast. In all cases the cultures were allowed to stand for approximately 1 min. after shaking with glass beads to permit the excess CO_2 to

dissipate. An attempt was made to correlate turbidity readings so made with the number of cells per milliliter but, because of the variability of this yeast in size and shape of cells (FIG. 1) the results of this attempt were considered to be valueless as an indication of the amount of growth of the organism. A Spencer bright-line haemocytometer was used for the cell counts.

Ester determinations in the yield studies were made by distilling approximately 25 ml. from a 50 ml. sample of fermentation medium,

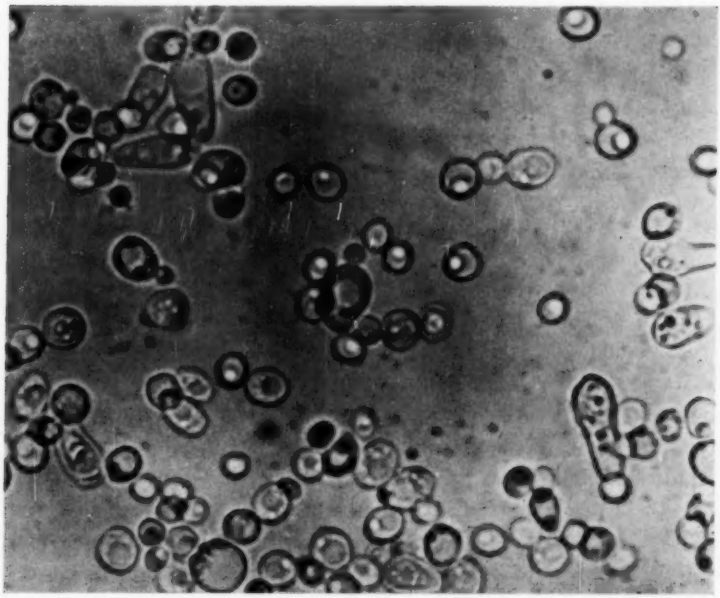

FIG. 1. Photomicrograph of *Hansenula anomala* showing differences in size and shape of cells.

adding five drops of phenolphthalein, adjusting the distillate with 0.1 *N* sodium hydroxide until it was just pink and refluxing for 1 hr. with 25 ml. of 0.1 *N* sodium hydroxide. Upon cooling, 25 ml. of 0.1 *N* hydrochloric acid was added and this was titrated with 0.1 *N* sodium hydroxide. The amount of sodium hydroxide used during saponification of the ester was equivalent to the sodium hydroxide used in this final titration. The amount of ethyl acetate

present in the original sample could then be calculated from this value. All ethyl acetate values are presented on the basis of milligrams per 100 ml. of medium.

Serial transfers were made to determine whether *H. anomala* would be able to live and continue to grow over an extended period of time in a substrate containing no added vitamins. The initial transfer was made by placing a standard loopful of inoculum in a French Square bottle containing 10 ml. of medium. Subsequent transfers were made by removing a standard loopful from the preceding 3-day-old culture. Growth was measured at the end of each 3-day interval by use of the colorimeter as in the other growth studies. Enough medium was made and bottled at one time to allow this experiment to be continued for 60 days. Aluminum foil was placed over the cotton stoppers of the bottles of sterile medium that were to be stored in the refrigerator to reduce evaporation.

RESULTS

Reference to TABLE 1 will show that the greatest difference in growth between the cultures containing no added vitamins and those containing added vitamins was apparent in the first 48 hr. after inoculation. At 96 hr. both sets of cultures contained approximately equal amounts of yeast. When casein hydrolysate was added to the medium there was an increase in growth over cultures containing no casein. The growth in cultures containing casein but no vitamins showed a rapid increase between 48 and 96 hr., while growth in cultures containing both vitamins and casein appeared to be leveling off at this time. When single vita-

TABLE 1

EFFECT OF ADDED VITAMINS AND CASEIN ON THE GROWTH OF *Hansenula anomala* (V = VITAMINS; C = CASEIN HYDROLYSATE)

Age of culture (hours)	Colorimeter readings			
	−V−C	−V+C	+V−C	+V+C
24	23.0	26.6	41.0	57.0
48	181.0	216.0	266.0	282.6
72	310.0	411.6	375.0	400.0
96	458.0	500.0	430.0	455.0

TABLE 2

EFFECT OF THE OMISSION OF SINGLE VITAMINS UPON THE GROWTH OF *H. anomala*

Type of culture medium	Colorimeter readings		
	Age of culture in days		
	2	4	6
−all	20	400	475
−riboflavin	53	450	450
−biotin + avidin	45	400	450
−biotin	65	410	450
−choline	120	410	450
−inositol	150	430	475
−*p*-aminobenzoic acid	260	450	475
−pantothenic acid	150	450	475
−pyridoxin hydrochloride	185	450	475
−niacin	135	380	400
−thiamin hydrochloride	120	380	475
+all	250	353	485

mins were omitted from the medium differences in the growth rate were apparent only in the first 24 hr. (TABLE 2). After that time the amount of growth in all of the cultures was approximately the same.

None of the cultures, with the exception of the culture containing thiamin, grew as well when a single vitamin was added (TABLE

TABLE 3

EFFECT OF ADDITIONS OF SINGLE VITAMINS ON THE GROWTH OF *H. anomala*

Type of culture medium	Colorimeter readings		
	Age of culture in days		
	2	4	6
−all	0	290	425
+riboflavin	35	435	495
+biotin	15	265	300
+choline	20	265	455
+inositol	35	265	330
+*p*-aminobenzoic acid	20	265	505
+pantothenic acid	35	265	405
+pyridoxin hydrochloride	70	435	455
+niacin	20	405	355
+thiamin hydrochloride	35	435	655
+all	165	435	515

3) as when all the vitamins were present in the medium. All grew better when they were cultured on these media than when they were cultured on media with no added vitamins.

It is apparent from TABLE 4 that the colorimeter readings of the serial transfers which were made at intervals of 3 days for a

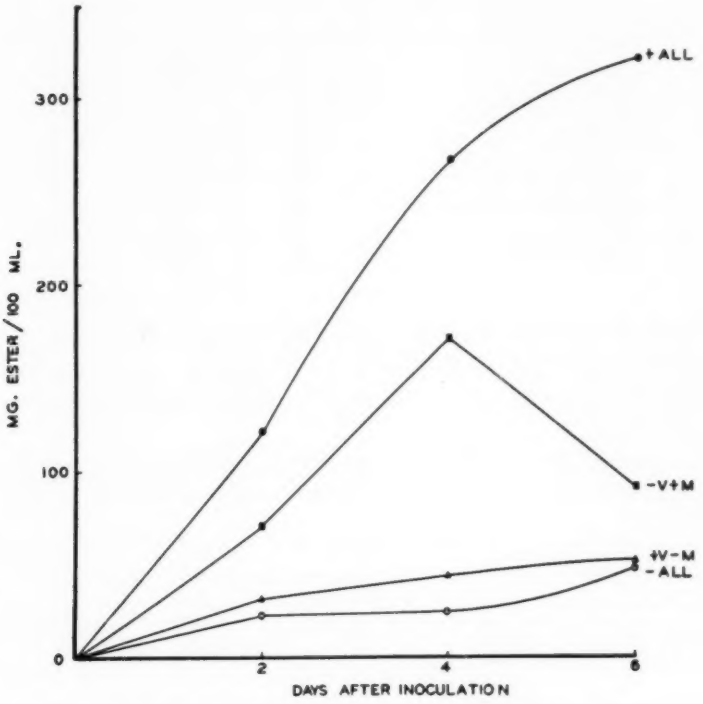

FIG. 2. The effect of vitamins (V) and trace elements (M) on ester synthesis by *H. anomala*.

period of 60 days indicated that the growth rate did not diminish when the yeast was cultured in basal medium to which no vitamins had been added. The colorimeter reading of the initial culture was 390 while the reading obtained from the final culture was 435.

Results of ester yield studies are shown in FIGS. 2 and 3. It is obvious that yeast cultured in medium containing both added vita-

mins and trace elements gave a higher overall yield of ethyl acetate than yeast cultured in any other medium used in these studies.

The production of ester in the cultures containing added vitamins and trace elements appeared to be leveling off about the sixth day after inoculation, but a sharp peak yield was never reached. Maxi-

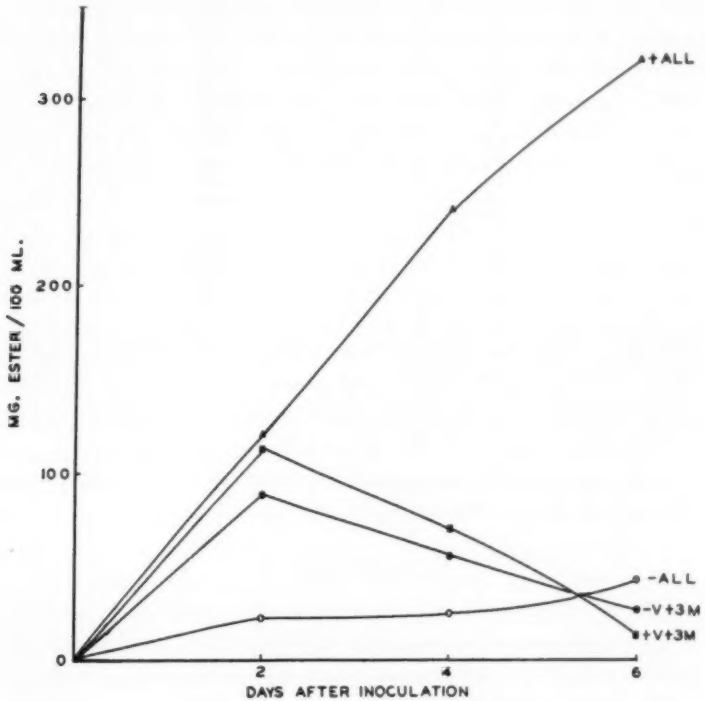

FIG. 3. The effect upon ester synthesis of the addition of trace elements in three times the usual concentration. (V = vitamins; M = trace elements.)

mum ester production was reached about the fourth day in the cultures containing only the usual concentration of trace elements after which the ester yield dropped sharply. When three times the usual amount of trace elements was added the yield rose quickly reaching a maximum the second day after inoculation in both the cultures containing vitamins and those containing no

TABLE 4

RESULTS OF AERIAL TRANSFERS MADE INTO BASAL MEDIUM WITH NO ADDED VITAMINS

Days	Colorimeter readings
3	390
6	520
9	500
12	530
15	460
18	480
21	530
24	480
27	520
30	520
33	460
36	410
39	460
42	285
45	460
48	560
51	460
54	360
57	435
60	490

vitamins. After the second day ester production dropped steadily. When vitamins were added but trace elements were omitted, yields were low over the entire course of the fermentation, reaching a slight peak of about 59 mg. of ester on the sixth day.

In no case was there a complete disappearance of the ester from the cultures as reported by Gray (*loc. cit.*).

DISCUSSION

On the basis of the experimental results obtained, *H. anomala* is apparently able to synthesize all of the vitamins of the B-complex that it utilizes. The increase in growth at the end of 96 hours when casein hydrolysate was added to the medium was probably due to the increased amount of nitrogen provided by the casein. In the presence of the vitamins the additional nitrogen-containing carbon compounds may be used in fermentation processes while in the absence of vitamins there is a shift to cell synthesis due to an inadequacy in certain enzyme systems necessary in fermentation.

The differences in growth during the first 24 hours of incubation are similar to the results obtained by Leonian and Lilly (**1942**) in their studies on ten strains of *Saccharomyces cerevisiae*. They found that when the incubation period was 48 hours a number of

yeasts manifested apparent dependence upon one or more added growth factors which did not seem necessary when the incubation period was extended to 72 hours.

In the yield studies it seems obvious that there is a definite interaction between the vitamins, trace elements, and the synthesis of ethyl acetate. The addition of trace elements alone increased the yield much more than the addition of vitamins alone, but when both were added the yields were the highest. The trace elements may well serve as activators or coenzymes of specific enzyme systems while the vitamins may complete others. Rogosa (**1944**) states that the vitamin requirements of certain yeasts may be associated with the production of certain fermentation enzymes although probably not in a causal relation.

In replications of the ester yield studies it was noticed that there was a considerable variation in the total yield of ester from run to run. Emphasis should be placed upon the fact that although this variation was quite common, the time after inoculation at which the maximum ester yields were reached was the same in all cases. In the studies of growth which were based on serial cultures (TABLE 4) there is considerable variation in some of the colorimeter readings from one reading to the next. It is possible that variations in the size of the inoculum from run to run may be partly the cause of some of the differences. Leonian and Lilly (*loc. cit.*) found that when 0.01 mg. of cells of *S. cerevisiae* constituted the amount of inoculum some yeasts grew very poorly; when this amount was increased growth increased correspondingly. On the other hand, *H. anomala* sporulates readily and it is possible that the cultures are comprised of an extremely heterogeneous population the individuals of which vary in all degrees in their ability to synthesize ester or to utilize the different vitamins and trace elements. According to Lindegren (**1945**), haplophase yeasts are nearly always inferior in their fermentative ability when compared quantitatively or even qualitatively to the diploid parent and many of them have lost certain specific characteristics. He cites an example of a single ascospore culture originating from *S. cerevisiae* that is unable to ferment sucrose, maltose, or galactose although the original diploid cells successfully fermented these sugars. In earlier studies Lindegren, Spiegelman, and Lindegren (**1944**) con-

cluded that while the genetic composition determines the initiation of enzyme syntheses, the quantitative level of enzyme in the cell below the maximum depends upon an interaction between the specific fermentable substrate and the cytoplasm. Subramaniam (**1948**) has obtained results from his studies on a strain of distiller's yeast indicating that fermenting yeast cells may become highly endopolyploid, thereby losing their capacity for vegetative reproduction. It seems that an increase or decrease in the numbers of this type of cell might be expected to affect the yield results.

SUMMARY

Investigations were conducted to determine the relation of nine vitamins of the B-complex and six trace elements to growth and ethyl acetate production by *Hansenula anomala* (Hansen) Sydow. Results of these investigations were as follows:

1. Twenty-four hours after inoculation into medium with added vitamins there was a decided increase in growth over the cultures containing no added vitamins. The greatest difference between cultures containing no vitamins and cultures containing all vitamins was apparent at about 48 hours. After 96 hours the amount of growth in these cultures was essentially equal. Addition of single vitamins resulted in similar growth differences. On the basis of these results it was concluded that this yeast has no vitamin deficiency for the nine vitamins of the B-complex that were tested.

2. The yields of ethyl acetate were highest when both vitamins and trace elements were added to the medium. An increase in the concentration of trace elements gave an increase in the amount of ester synthesized.

3. There was a greater increase in ethyl acetate production when trace elements alone were added to the medium than when vitamins were added alone.

4. An interaction between vitamins, trace elements, growth, and ester production by this organism is indicated by these studies.

DEPARTMENT OF BOTANY,
THE OHIO STATE UNIVERSITY,
COLUMBUS 10, OHIO

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STUDIES ON THE NUTRITION OF MORCHELLA ESCULENTA FRIES [1]

THOMAS D. BROCK

(WITH 1 FIGURE)

Morchella esculenta, the common morel, is highly prized for its culinary aspects. It has an excellent flavor, reputed to be superior to that of the mushroom of commerce, *Agaricus campestris*. This would make it highly desirable to obtain methods of cultivation, but fruiting bodies have rarely been reported to occur in culture, and never, to the author's knowledge, in pure culture. Two different workers have reported obtaining morels in culture. Repin (**1901**) claims to have obtained fruiting bodies in 1901 in a cave in which cultures had been established in flower pots in 1892. He also got fruiting bodies around a bed composed of dry leaves rendered alkaline with sodium carbonate and in a trench in which an apple residue had been deposited. The inoculum which he used was mycelium that had been grown in long, narrow tubes for many months and then transferred to the various soils. He established in his work that the morel does not necessarily grow parasitically on roots of trees and can grow saprophytically. He comments that it should be possible to establish conditions for the commercial production of morels.

The other work on culture of morels is that of Molliard (**1904**, **1905**). In 1904, he reported the conidial stages of *M. esculenta* var. *rotunda*, *M. conica*, and *M. deliciosa*. The conidia formed on a compost to which he had added various organic substances, although he does not state what these substances were. The conidial stage is apparently identical with the genus *Costantinella*, described

[1] Papers from the Department of Botany and Plant Pathology, Ohio State University, No. 526. The work reported in this paper is taken from a thesis submitted to the Graduate School of the Ohio State University in partial fulfillment of the Degree of Master of Science. The author expresses his thanks to Dr. W. D. Gray, under whom this work was done.

earlier occurring on leaf mold. The conidia did not germinate. In another paper (**1905**), Molliard reports the formation of sclerotia of *Morchella* on sterile, moistened bread. These sclerotia sometimes grew rather large and Molliard said they looked and tasted like fruiting bodies, although he never observed ascus formation. He got true fruiting bodies on five kilograms of a *"compote de pommes."* The conidial stage also developed on this medium. Molliard does not state whether these fruiting bodies occurred on sterile media or not. He suggests a possible cultural practice: bury rotting fruits in soil and introduce the mycelium of the desired species in the fall, come back in the spring and harvest the fruiting bodies. Whether this works or not, it has surely not come into general use.

Costantin (**1936**) has reviewed Molliard's work together with some other papers on morel culture, and has published two pictures of fruiting bodies obtained in culture by Molliard.

Little work has been published on the physiology of *Morchella*. Fron (**1905**) has reported briefly on the utilization of a few carbon compounds and on the effect of acidity and alkalinity. Although he gave no quantitative data, he stated that inulin was the best carbon source and that a neutral or slightly alkaline reaction favored growth. He also did some work on requirements of the major minerals but his results probably should be discounted in view of the later work by Steinberg (see Foster, **1949**, for a discussion of his work), as it is almost certain that he did not have purified chemicals. Melin (**1941**) stated that thiamin has no effect on the growth of unisporic cultures of *M. conica*.

Roze (**1883**) reported that *M. esculenta* was found parasitizing the rhizomes of the Jeruselum artichoke, *Helianthus tuberosus*. Jeruselum artichoke is known to have a high content of the polysaccharide inulin.

Although fruiting bodies are difficult to obtain in culture, the mycelium of *M. esculenta* grows rather well on a synthetic medium. On agar and in liquid culture it forms a white mycelium which gradually turns brown. Sclerotia form in agar culture. In the present work no spores have ever been noticed in culture, although in view of Molliard's work their formation is possible. Since little

is known about the physiology of the fleshy Ascomycetes, and because of the general interest in *Morchella* as an edible fungus, it seemed desirable to carry out the present studies.

MATERIALS AND METHODS

The mycelial culture of *M. esculenta* was originally isolated by W. D. Gray from stipe tissue of a fruiting body collected near Ames, Iowa, in 1946. Stock cultures have been maintained since that date on glucose-yeast extract-KH_2PO_4 agar medium. The basal mineral salts medium used in the physiological studies was a modified Czapek-Dox medium of the following composition:

$MgSO_4 \cdot 7H_2O$	0.5 g.
K_2HPO_4	1.0 g.
$FeSO_4$	0.01 g.
Water, dist.	1000 ml.

Carbon sources were added in the amount of twelve grams of carbon per liter, nitrogen sources at the level of 250 milligrams of nitrogen per liter.

In the early experiments, the organism was cultured in long glass tubes half full of the agar media, and growth rate was measured by the daily advance of the mycelial frontier along the tube. This is essentially the technique described by Beadle and Tatum (**1941**) and Ryan, Beadle and Tatum (**1943**), although it has been used earlier in slightly different form and for a different purpose by Fawcett (**1925**) and Repin (**1901**). It is interesting that although Fawcett apparently developed this technique independently, Repin had used it twenty-four years earlier.

This technique did not prove entirely satisfactory for measuring the effect of nutrition on growth in *M. esculenta*, since differences which showed up in dry weight measurements on liquid cultures were not nearly as evident when using the tube method. (Compare TABLE 1 and TABLE 2.) It is thought that this might be due in part to the slower growth which *M. esculenta* makes as compared with *Neurospora*, an organism that is better adapted to the tube technique. A twenty mm. length increment per day was the most rapid growth obtained in *M. esculenta* as compared with a maximum of 96 mm. per day for *Neurospora*. In addition to growing in length over the agar, *M. esculenta* also grows up into

the air, sometimes making thick tufts of mycelium, sometimes not, depending upon the medium, and these differences do not show up when growth in length is the only criterion. It is evident from the present work that although the tube technique is often useful in certain types of physiological work, in other types it is highly inaccurate.

In most of the work, liquid cultures containing thirty ml. of medium per 125 ml. flask were used. The flasks were incubated without shaking. The sugar solution was sterilized separately from the mineral salts and added aseptically. Fifteen ml. of sterile double strength sugar solution, adjusted to a pH of 7 before autoclaving, was added to fifteen ml. of sterile double strength mineral salts solution. The pH of the mineral salts solution was adjusted with hydrochloric acid or sodium hydroxide before autoclaving and the final measurement was made with a Beckman Model H pH Meter after the sugar solution had been added. This pH was designated the initial pH. Neglect in measuring the pH of control flasks after autoclaving may result in erroneous conclusions in regard to the pH range for growth. It was found in the present work that the pH of unbuffered Czapek-Dox solution autoclaved with glucose at any pH above 7.0 dropped to values below 7.0. For example, the pH of media initially set at 11.0 dropped to 5.85. This large drop in pH is probably due to the formation of decomposition products of glucose, many of which are acids.

For inoculum, disks four mm. in diameter were cut from a rapidly growing petri plate culture grown on the basal medium plus glucose, sodium nitrate, and two per cent agar. One of these cylinders was used to inoculate each flask. In this way, the inocula sizes were kept approximately uniform.

As there were no facilities available for incubation at constant temperature, all flasks were incubated at room temperature. This lack of temperature control undoubtedly limits the amount of comparison which can be made between experiments conducted at different times, but different treatments within one experiment can be compared readily. Six days' incubation was found to be satisfactory and each experiment was terminated at the end of this time. The mycelial mats were removed from the flasks with a glass rod, squeezed to remove excess moisture, and placed in tared glass vials.

This technique is much faster and more convenient than filtering because the vials can be washed out and used over without reweighing. It is possible to use this technique, of course, only when the mycelium of the species of fungus used is one that does not fragment during growth in liquid culture. The mycelial mats were dried to constant weight at 90° C. In all experiments, the dry weight values represent the average of at least three separate determinations.

EXPERIMENTAL RESULTS

Utilization of carbon compounds.—In TABLE 1 are listed the dry weights of mycelium obtained on twenty-six different media which varied only as to the nature of the carbon compound. Soluble starch, maltose, fructose, and turanose all appear to be better than glucose, although soluble starch is the only one that differs significantly, as shown by the "t" test. Inulin is obviously a poor

TABLE 1

GROWTH OF *M. esculenta* ON DIFFERENT CARBON COMPOUNDS ADDED TO THE BASAL MEDIUM IN THE AMOUNT OF 12 GRAMS OF CARBON PER LITER. DRY WEIGHTS ARE IN MILLIGRAMS AND REPRESENT THE AVERAGE OF THREE SEPARATE DETERMINATIONS

Carbon source	Mg. dry weight
Starch	79
Maltose	71
d(−)Fructose	48
d(+)Turanose	45
d(+)Glucose	34
Sucrose	31
d(+)Raffinose	23
d(+)Mannose	23
d(+)Galactose	21
l(+)Arabinose	21
Cellobiose	20
d(+)Trehalose	15
d(+)Xylose	11
Inulin	7
l-Sorbose	7
d-Mannitol	6
d(+)Melibiose	6
Adonitol	6
Lactose	6
d-Sorbitol	6
l(+)Rhamnose	5
d(−)Lyxose	4
l(−)Fucose	4
d(+)Melezitose	3
d(−)Arabinose	2
Dulcitol	2
Check	1

TABLE 2

GROWTH IN LENGTH OF *M. esculenta* ON AGAR IN LONG TUBES WITH DIFFERENT CARBON COMPOUNDS ADDED TO THE BASAL MEDIUM IN THE AMOUNT OF 12 GRAMS OF CARBON PER LITER. NITROGEN SOURCE, SODIUM NITRATE. FIGURES REPRESENT GROWTH INCREMENT IN FOUR DAYS

Carbon source	Growth in mm.
Sucrose	86
d(−)Glucose	84
Maltose	83
d(−)Fructose	80
Starch	78
d(−)Mannose	77.5
d(−)Raffinose	77
l(−)Arabinose	75
d(−)Xylose	73
Inulin	72
d(−)Galactose	68
Cellobiose	61
d-Sorbitol	52
Lactose	7
Dulcitol	—

carbon source. All of the sugar alcohols tested, as well as the methyl sugars (rhamnose and fucose) are considerably less available than the sugars from which they are derived. The 5-carbon sugars which were tested are less available than glucose. It is interesting that l (+) arabinose, the naturally occurring isomer, is considerably better than d (−) arabinose. Although the disaccharide maltose is utilized rather well, the two other glucose-glucosides tested, trehalose and cellobiose, which have different linkages between the two glucose units, are poorer carbon sources. The glucose linkage in maltose is the same as that in starch (alpha, 1–4). Other disaccharides that are poorly used are melibiose and lactose, both glucose-galactosides. Of the two trisaccharides tested, raffinose (galactose-glucose-fructoside) is intermediate and melezitose (glucose-fructose-glucoside) is extremely poor. Sorbose was much less available than fructose, although both are 6-carbon ketoses.

Since the results with starch seemed rather unusual, it was thought that this might be due to growth-promoting substances introduced with the starch. That starch is high in impurities is well known (Schoch, in Gortner, **1949**). Growth-promoting substances in soluble starch have been reported by Ryan, Beadle and Tatum (**1943**) for *Neurospora*, and were partially removed by

them with activated carbon. Consequently, an attempt was made to purify the soluble starch used in the present work with activated carbon (Norit A). No decrease in growth was observed in Norit-treated as compared with untreated starch. In fact, treated starch was a little better than untreated starch for growth, although the difference was not statistically significant. It is possible that the soluble starch contains some growth factor(s) not removed by the carbon, although this point has not been further investigated. The soluble starch used was the Coleman and Bell product, which is rendered soluble by treatment with acid (William H. Bell, personal communication); obviously, the exact nature of its molecular configuration is not known.

The results of some initial experiments on carbon sources, using the tube technique, are given for comparison with those in liquid culture (TABLE 2). Although these results are subject to the limitations previously pointed out, there is no doubt that inulin serves as a good carbon source when the fungus is cultured on agar, as opposed to the almost negligible growth in liquid culture.

Utilization of nitrogen compounds.—In TABLE 3 the dry weights obtained on thirty-four media varying only as to the nature of the nitrogen compound are listed. In this series, replicates of four were used instead of the usual three, except in certain instances where contamination occurred. The pH was adjusted to 7.0 before autoclaving and measured on control flasks after autoclaving. In general, the pH range between different series varied between 6.5 and 7.0 with the exception of the hydrazine, tryptophan and urea series which varied from this range as indicated.

The best growth occurred on cysteine-HCl, but as this compound decomposed at least partially upon autoclaving (H_2S gas was evolved), it is hard to say whether the intact molecule or some decomposition product was used. Aspartic acid and asparagine were almost as good nitrogen sources as cysteine-HCl. Several of the ammonium salts and urea were just slightly less effective than these, although ammonium citrate was highly toxic. Sodium nitrite was better for growth than sodium nitrate. Quite a few of the nitrogen compounds yielded poorer growth than the controls. It cannot be stated definitely whether some of these are toxic or not, but it is fairly certain that ammonium citrate, thiourea, hydroxyl-

amine-HCl and hydrazine-2HCl are. The pH of the hydrazine series was 5.1, however, and this might have been a factor in its toxicity. The toxicity of thiourea is interesting, since the substitution of a sulfur atom for an oxygen atom seems to make the difference between inhibition and good growth.

In general, the pH changes during incubation were dependent upon the type of nitrogen compound present and the extent of growth. With basic amino acids (*e.g.*, tryptophan) and ammo-

TABLE 3

GROWTH OF *M. esculenta* ON THE BASAL MEDIUM WITH GLUCOSE AS CARBON SOURCE AND DIFFERENT NITROGEN SOURCES ADDED IN THE AMOUNT OF 250 MG. OF NITROGEN PER LITER. THE DRY WEIGHT FIGURES REPRESENT THE AVERAGES OF AT LEAST THREE DETERMINATIONS IN EACH SERIES

Nitrogen source	Initial pH	Final pH	Mg. mycelium per culture
l-Cysteine-HCl	6.9	5.85	35
dl-Aspartic acid	6.65	7.0	32
l(+)Asparagine	6.75	6.6	30
Urea	7.6	7.05	26
Sodium nitrite	7.0	7.3	25
Ammonium tartrate	6.5	5.2	25
Ammonium nitrate (normal concentration	6.75	4.9	25
Ammonium nitrate (2× norm. conc.)	6.65	4.2	24
dl-alpha-alanine	6.9	6.4	21
Ammonium chloride	6.7	5.0	21
Ammonium sulfate	6.65	4.5	20
l-Glutamic acid-HCl	6.5	7.1	20
Sodium nitrate	7.0	7.3	17
dl-Histidine-HCl	6.75	6.65	15
dl-Leucine	6.9	6.4	15
l-Ornithine	6.8	6.6	14
dl-Valine	6.85	6.55	12
dl-Methionine	6.9	6.5	11
dl-Tryptophan	7.25	6.95	10
dl-Serine	6.85	6.75	9
l-Taurine	6.8	6.8	9
dl-Proline	6.85	6.8	8
Check (no N compound added)	7.0	6.9	7
Glycyl glycine	6.75	6.7	7
dl-Threonine	6.85	6.8	7
Acetamide	6.9	6.85	6
Glycine	6.95	6.9	6
Creatine	6.9	6.9	5
dl-Lysine-HCl	6.8	6.7	5
dl-Sarcosine-HCl	6.9	7.0	4
Ammonium citrate	6.6	6.6	2
Thiourea	6.9	6.9	1
Hydroxylamine-HCl	6.85	6.6	0
Hydrazine-2HCl	5.1	5.1	0

TABLE 4

GROWTH OF *M. esculenta* ON GLUCOSE-SODIUM NITRATE BASAL MEDIUM AT DIFFERENT pH VALUES. DRY WEIGHT VALUES REPRESENT THE AVERAGE OF THREE SEPARATE DETERMINATIONS FOR EACH TREATMENT

Initial pH	Final pH	Mg. mycelium per culture
2.3	2.2	0
3.1	3.1	2
4.45	6.95	41
5.23	7.3	71
6.0	7.4	81
6.93	7.7	96
7.45	7.7	66
8.3	7.8	78
10.1	9.2	1
11.18	9.5	1

nium salts, the pH dropped during incubation, probably due to the differential absorption of the basic group from the medium. With acidic amino acids (*e.g.*, aspartic acid) and sodium nitrate and nitrite, the pH rose, probably due to the differential removal of the acidic group. In the ammonium nitrate series, the pH dropped, indicating the differential absorption and/or utilization of the ammonium ion over the nitrate ion.

In the tryptophan series, a yellowish brown, water-soluble pigment was observed in the culture medium. This had been previously noticed in tube cultures on agar. The pigment is also soluble in ethyl alcohol (95%), but insoluble or only slightly soluble in diethyl ether or acetone. Although the pigment has been precipitated from alcoholic solution with diethyl ether, no physical constants have as yet been determined. The pigment also forms

TABLE 5

GROWTH OF *M. esculenta* ON GLUCOSE BASAL MEDIUM AT DIFFERENT pH VALUES WITH AMMONIUM CHLORIDE OR SODIUM NITRITE AS NITROGEN SOURCE. DRY WEIGHT VALUES REPRESENT THE AVERAGE OF THREE SEPARATE DETERMINATIONS FOR EACH TREATMENT

	Initial pH	Final pH	Mg. mycelium per culture
NH_4	3.1	3.1	2
	5.0	2.95	24
	6.7	4.28	21
	8.4	7.6	27
NO_2	4.7	7.05	50
	5.8	6.45	11
	7.05	7.4	33
	8.4	7.55	33

a water-insoluble product with 2,4-dinitrophenylhydrazine. Further studies on this pigment are in progress.

A reddish water-soluble pigment was also noticed in the histidine series, but as yet has not been further investigated.

Relation of pH to growth.—In TABLE 4 are listed the dry weights obtained with *M. esculenta* when cultured on the basal medium with glucose and sodium nitrate at different initial pH values. These data have been presented graphically in FIGURE 1. There is a double maximum in the growth curve, with peaks at 7.0 and 8.3 and a minimum at 7.5–7.7. In the table, both the initial pH and the final pH after incubation are listed.

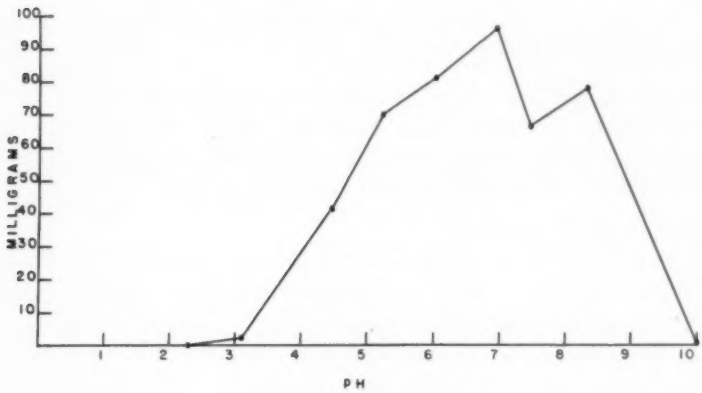

FIG. 1. *Morchella esculenta.*

In TABLE 5 are the dry weight values obtained when ammonium chloride and sodium nitrite were used as nitrogen sources. Since only four pH values were used, accurate growth curves cannot be drawn. The high yield obtained at pH 4.7 with sodium nitrite is probably due to the fact that at this low pH value, nitrite is oxidized to nitrate during autoclaving and thus there is a reduction in the amount of the toxic free nitrous acid. It is a known chemical fact that such an oxidation occurs (Ephrains, **1943**).

The pH changes which occurred during incubation are interesting. With the ammonium salt, the pH dropped during incubation, no matter what the initial pH value was. In the sodium nitrate

TABLE 6

GROWTH IN LENGTH OF *M. esculenta* ON GLUCOSE-BASAL MEDIUM AND AGAR WITH DIFFERENT NITROGEN SOURCES ADDED IN THE AMOUNT OF 250 MG. OF NITROGEN PER LITER. FIGURES REPRESENT GROWTH INCREMENT IN FOUR DAYS

Nitrogen source	Growth in mm.
Sodium nitrate	69
Urea	43
Glycine	39
Proline	20
Serine	16
Tryptophan	7
Alanine	++
Leucine	+
Valine	+
Check	+
Ammonium chloride	−
Sodium nitrite	−

Legend: − no growth, + growth noticeable but not measurable, ++ growth a little better than checks.

series, the pH rose when the initial value was below 7.5–7.7 and dropped when the pH was above this value. This pH corresponds to the pH at the minimum point on the pH-growth curve; pH changes in sodium nitrite medium were similar to those in sodium nitrate medium.

In some of the early experiments, the tube technique was used. These experiments were run before the optimum pH had been determined and were conducted at pH 5.5. The results are listed in TABLE 6. It is obvious from these data that both ammonium and nitrite are toxic under these conditions. No growth at all occurred with either of these nitrogen sources. Glycine, on which the fungus hardly grew at all in liquid culture, was used rather readily in growth on agar. Alanine, on which the fungus grew rather well in liquid culture, supported scant growth on agar.

As a further test of the ammonium and nitrite toxicity on agar, these compounds were added separately to media containing sodium

TABLE 7

GROWTH OF *M. esculenta* ON THE BASAL MEDIUM PLUS GLUCOSE, SODIUM NITRATE, AGAR, AND THREE MINERAL SALTS AS NOTED. GROWTH IS DENOTED BY + AND NO GROWTH BY −

Equimolar amts. of	Growth
$NaNO_3 + NaCl$	+
$NaNO_3 + NH_4Cl$	−
$NaNO_3 + NaNO_2$	−

nitrate in the usual concentration (TABLE 7). Both ammonium and nitrite are extremely toxic under these conditions at the pH used (5.5). This is in marked contrast with the results reported above for ammonium and nitrite in liquid cultures at pH 5.0 and 5.6, respectively. It would appear that the fact that the fungus is growing on agar is a factor in this toxicity.

DISCUSSION

In the study of the relationship of nutrition to growth, it must be kept in mind that no single factor in itself determines the extent of growth, but that a complex of factors, all more or less interrelated, is involved. Since any attempt to elucidate carbon and nitrogen requirements of a fungus necessarily involves the control of all variables except the carbon or nitrogen compounds involved, any interrelationships between the two are overlooked. In that respect, the experiments reported in the present paper are limited in scope. However, if it is always kept in mind that the carbon requirements have all been determined with nitrate as nitrogen source, and the nitrogen requirements have been determined with glucose as carbon source, useful information is still obtainable. In the following discussion, any interpretations advanced are considered valid only under the particular set of conditions used, and no attempt is made to generalize these interpretations to all cases.

Since no assay for residual sugar was made at the end of the growth period, it is impossible to say whether carbon sources that yielded poor growth did so because of slower rate of utilization or because of less efficient assimilation of the carbon compound. It is possible that if the fungus had been allowed to grow for longer periods of time on sugars on which it appeared to be growing poorly, it might have attained dry weight values as high as it did on sugars that appeared to be more readily utilized.

Starch, maltose, fructose, turanose, glucose, and sucrose all yielded high dry weight values. In an intermediate class were raffinose, mannose, galactose, arabinose, and cellobiose. The rest of the carbon sources, including the polyhydric alcohols and the methyl sugars, yielded rather low dry weight values. In the only other nutritional work reported for *Morchella*, Fron (**1905**), using

liquid cultures, found little or no growth on sucrose, fructose, or mannitol, but abundant growth on glucose, invert sugar, inulin, or starch. The present results do not agree entirely with his. The differences could be due to strain differences or to differences in culture media. As a basal medium Fron used Raulin's solution while the basal medium used in the present studies was a modified Czapek-Dox solution.

Structural differences between sugars have been used to explain differences in growth of fungi on various sugars by Cantino (**1949a**, **1949b**) and Steinberg (**1942**). This type of interpretation can also also be used in the present work. The 6-carbon aldoses, glucose, mannose and galactose, are much better utilized than their corresponding alcohols, sorbitol, mannitol, and dulcitol. Apparently the aldehydic or reducing group of the sugar is necessary for utilization to be effected. The 5-carbon sugars, arabinose, and xylose, yielded lower dry weight values than the 6-carbon sugars. That optical activity is important in utilization is shown by the data for arabinose, the only sugar for which both isomers were available. The $l(+)$ form of arabinose is utilized while the $d(-)$ form is not.

Apparently the alpha, 1–4 linkage between glucose units is easily broken, since starch and maltose, which contain this linkage, yield high dry weight values. However, the beta, 1–4 linkage of cellobiose is probably not as easily broken, since dry weight values obtained with this sugar were only one-third as high as those obtained with maltose. This is also true for the alpha-1, alpha-1 linkage of trehalose. It is possible, however, that starch and maltose contain growth-affecting impurities which may account for the high dry weight values on these sugars. Apparently the glucose-fructose linkages of sucrose and turanose are easily broken. However, the galactose-glucose linkage in both melibiose and lactose is broken with difficulty as is evidenced by the almost negligible growth on these sugars. Lactose seems to be a poor carbon source for many fungi (Cantino, **1949a**; Michener and Snell, **1950**; Baker and Smith, **1942**; Goddard, **1934**; Ryan, Beadle and Tatum, **1943**). However, Perlman (**1948**) found lactose to be a good carbon source for *Memnoniella*.

The utilization of the trisaccharides is interesting. Raffinose

which contains one linkage as in sucrose and another as in melibiose was utilized fairly readily. Since the melibiose linkage is difficultly broken (see above), it is probably the sucrose linkage which is broken in raffinose. Melezitose, the other trisaccharide tested, was a very poor carbon source. Interestingly enough, it contains a sucrose and a turanose linkage, both of which are rather readily broken in disaccharides (see above). It is possible that entrance into the fungus is a factor in the poor utilization of melezitose.

Inulin has proved to be a very poor carbon source in these studies when the fungus is grown in liquid culture. This is unusual in view of the fact that Fron (**1905**) reported inulin to be a very good carbon source. *M. esculenta* has also been found parasitizing the rhizomes of *Helianthus tuberosus* (Roze, **1883**), which are high in inulin content. It is possible that strain differences might account for these discrepancies, but it is also possible that the difference in Fron's culture conditions or the purity of his inulin might be factors. Also, *H. tuberosus* rhizomes might contain some growth factor that enables the organism to utilize inulin. An attempt should be made to culture the fungus on extracts of *H. tuberosus* rhizomes. It should be pointed out that in the present work inulin was a rather good carbon source in agar cultures (TABLE 2). The agar used was unpurified and might possibly contain growth factors (Robbins, **1939**).

In no nutritional studies do the interactions of the environmental complex have more importance than in studies of nitrogen assimilation. Such factors as pH (Foster, **1949**), redox potential (Robbins, **1937**), presence of trace elements (Steinberg, **1939**), and aeration (Burk and Horner, **1939**) all may play a part in determining whether a particular nitrogen compound can be utilized. Robbins (**1937**) has also pointed this out and cited it as a limitation to his classification of plants based on nitrogen assimilation.

M. esculenta could be classified in Robbins' Group II, which includes organisms that utilize nitrate, ammonium, and organic nitrogen. However, only a limited number of the amino acids tried could be used to any extent for growth. Cysteine-HCl, aspartic acid, alanine, and glutamic acid were the only ones which were utilized effectively. Of these, all except cysteine are known to

occur in transamination reactions, and it is probable that this explains why good growth is realized on them. Unfortunately, the cysteine used was the levorotatory form while the other three were racemic mixtures of the *d*- and *l*-forms. It is possible that aspartic, glutamic, and alanine would have yielded as high dry weight values as cysteine if they had been used strictly as the *l*-form. It is interesting that serine, which differs from alanine only in the substitution of an hydroxyl group for a hydrogen atom on the beta carbon atom, yields much lower dry weight values than alanine.

Ammonium compounds or compounds which readily yield ammonia, such as urea and asparagine, are excellent nitrogen sources. Ammonium is probably used preferentially over nitrate, as is evidenced by the pH changes in the ammonium nitrate series; the drop in pH indicates preferential absorption of the basic ammonium group.

That nitrite is a better nitrogen source than nitrate is interesting because of the fact that it is usually found to be toxic for most fungi in the concentration used here. This toxicity probably occurs because the pH at which fungi are usually culture is in the acid range, where nitrite is known to be toxic (Cochrane and Conn, **1950**, Cochrane, **1950**, Nord and Mull, **1945**). At acid pH values, nitrite is generally in the form of undissociated nitrous acid. The undissociated form of an acid is known to have the greatest biological activity (Simon, **1950**). *M. esculenta*, as opposed to most fungi, grows best when the reaction of the medium is about at the neutral point, where nitrite would not be toxic. It is logical that nitrite should be as good as or better than nitrate, since nitrate probably is reduced to nitrite before it is further assimilated.

The toxicities of hydroxylamine, hydrazine, thiourea, and ammonium citrate were not entirely unexpected. Hydroxylamine, hydrazine, and thiourea are known enzyme inhibitors (Sumner and Somers, **1947**, Lardy *et al.*, **1949**), and citrate is a strong chelating agent and ties up many of the mineral elements (Hutner *et al.*, **1950**).

As in the case of carbon sources, it is impossible to tell whether a nitrogen source was poor because of slow rate of utilization or because of less efficient utilization. An indication that not all of the nitrogen source is used up within the experimental period (six

days) is given by the data on ammonium nitrate at two different concentrations (TABLE 3). No significant differences in dry weights were obtained at these two concentrations, indicating that all of the nitrogen had not been utilized in the medium of lowest concentration. However, the larger drop in pH in the 2 × usual concentration indicates that more of the nitrogen was absorbed by the fungus from this medium than from the medium of usual concentration.

Another example of the importance of environmental factors in nitrogen assimilation is indicated by the data on nitrogen assimilation on agar cultures (TABLE 6). Glycine is a good nitrogen source and alanine a poor one. In liquid cultures the reverse is true.

The formation of pigment in the culture medium when the fungus is grown on tryptophan is interesting. Pigments in several of the insects are known to be formed from tryptophan (Hirata, Nakanishi and Kikkawa, **1950**) and it is possible that the pigment formed by *M. esculenta* is similar to these. It would seem reasonable that the brown pigment in *M. esculenta* mycelium might be identical with the pigment which occurred in culture fluids containing tryptophan and possibly the biochemical steps in its synthesis could be worked out.

Hydrogen-ion concentration of culture media can affect the growth of a fungus in two ways: externally it can control the degree of dissociation of inorganic ions in the culture solution, and since dissociation plays a part in the movement of ions into the fungus, degree of dissociation will affect fungus growth; internally, it can affect the hydrogen-ion concentration in the cells themselves, causing changes in pH in the mycelium. The internal effect is probably the most pronounced. This internal effect of pH seems to be mainly on the cell colloids, causing changes in their charge. The effect of pH on permeability is probably through effect on cell colloid charges. This whole subject has been discussed by Foster (**1949**) (see also Scott, **1929**).

Evidence for this internal effect in *M. esculenta* is the relationship between pH and growth on sodium nitrate medium. The pH-growth curve (FIG. 1) rises to a maximum at 6.95, drops to a minimum at 7.45, and rises to another maximum at 8.3. Above

pH 8.3, the curve drops off very rapidly. This type of curve, which is bimodal, has been found in several other fungi (Hopkins, **1922**; Lindfors, **1924**; Tilford, **1936**; Webb, **1919**; Webb, **1921**). The minimum point has been interpreted by Scott (**1929**), Robbins and Scott (**1925**), and Tilford (**1936**) as the pH corresponding to the isoelectric point of ampholytic colloids. Whether this refers to the fungus colloids as a whole or only to membrane colloids is not certain. This same phenomenon of colloidal isoelectric points has been demonstrated by Bünning (**1936**) using acidic and basic dyes at different pH values. In this work, basic dyes were absorbed at pH values above 3.1 and acid dyes at pH values below 3.1, pH 3.1 thus being the isoelectric point of cell colloids for *Aspergillus niger*. As far as the author has been able to determine, all of the isoelectric points found so far for fungi have been on the acid side of neutrality, usually pH 6.0 or below. The isoelectric point for *M. esculenta* appears to be around pH 7.5.

M. esculenta has an optimum pH for growth at 7.0. This also is unusual, as it is a well known fact that most fungi have their optima in the more acid range (Wolf and Wolf, **1947**), although Cross (**1948**) found the optimum pH for *Histoplasma capsulatum* to be 7.2–7.6 and Wolf, Bryden and McLaren (**1950**) found *Monosporium apiospermum* to have an optimum at 7.0–7.6. Both of these fungi are human pathogens. However, Fron (**1905**) also found that *Morchella* grew best in a neutral or slightly alkaline medium.

Although the data on effect of pH on growth in media containing nitrite and ammonium as nitrogen sources are limited, it appears that optima here are above pH 7.0.

pH changes during incubation also illustrate several interesting points. In the media containing sodium nitrate and sodium nitrite, media with reactions on the acid side rose in pH, while the media on the alkaline side dropped in pH. The pH changes seem to approach 7.7 in the sodium nitrate series, which is very close to the pH at which the minimum point on the growth curve appears. There is insufficient data in the sodium nitrite series to be positive of the value here, but it is probably about the same as in the nitrate series. This pH change, due undoubtedly to the differential absorption of acidic and basic groups, is characteristic of fungi when

cultured in solutions differing in pH from their isoelectric points, provided organic acids are not elaborated which would mask the pH changes (Scott, **1929**). At the isoelectric point there is equal absorption of acidic and basic groups and the pH does not change. This, then, is another indication that the isoelectric point of cell colloids of *M. esculenta* is in the region 7.5–7.7.

In the ammonium series, however, the pH drops upon incubation, no matter what the original pH was. It would be expected, if the same phenomenon of differential absorption occurred with ammonium salts, that media of acid pH would become more alkaline. Whether this difference in the nature of pH changes with ammonium is indicative of a different type of absorptive mechanism than for that of nitrite and nitrate is uncertain.

Aeration might also affect the utilization of nitrogen compounds. Further work should be carried out along these lines.

SUMMARY

There has been very little work done on the physiology of fleshy Ascomycetes. *Morchella esculenta,* because of its possible role as a food mushroom, is of much general interest and it would be highly desirable to have basic nutritional studies done on it. The present paper presents the beginnings of such a study.

M. esculenta will grow on agar or in liquid culture on synthetic media composed of mineral salts and suitable carbon or nitrogen sources. A survey of many such carbon and nitrogen sources was made. The organism grew well on starch, maltose, fructose, turanose, glucose, and sucrose and moderately well on several others. Polyhydric alcohols and levorotatory methyl sugars supported little growth. Inulin was utilized in growth on agar but not in liquid culture.

As nitrogen sources, cysteine-HCl, aspartic acid, asparagine, urea, sodium nitrite, various ammonium salts, alanine, glutamic acid-HCl, and sodium nitrate were favorable. Other amino acids and nitrogen compounds were intermediate or poor. Ammonium citrate, thiourea, hydroxylamine-HCl, and hydrazine-2HCl were toxic.

The pH-growth curve for this organism was bimodal, with maxima at pH 6.93 and 8.3 and a minimum at 7.45. Optimum pH

for growth was at pH 6.93, which is unusually high for fungi. The bimodal curve is attributed to the effect of pH on the fungus isoelectric point; the minimum point at pH 7.45 is interpreted as the isoelectric point for the cell colloids.

Apparently pH, carbon source, and other factors influence the utilization of nitrogen compounds.

These results are discussed from the standpoint of the experimental techniques required in a nutritional study and of the various general aspects of fungus physiology.

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STUDIES OF AN INSECT MYCOSIS. II. HOST AND PATHOGEN RANGES [1]

ALFRED S. SUSSMAN

In a previous paper (Sussman **1951**), it has been shown that *Aspergillus flavus* Link can parasitize each stage in the life history of the cecropia moth, *Platysamia cecropia* L. These observations have now been extended by an investigation of the host and parasite ranges of these organisms.

PATHOGEN RANGE

To investigate the possibility that Aspergilli other than *A. flavus* might parasitize *P. cecropia*, at least one representative of each of the "groups" of Aspergilli delimited by Thom and Raper (**1945**), except for the *A. candidus* group, was used as inoculum. Also, genera related to *Aspergillus*, like *Penicillium* and *Paecilomyces* were included in these tests, as were miscellaneous other animal and plant pathogens.

Inoculation was accomplished by injecting 0.05 ml. of a heavy spore suspension of each of the species listed in TABLES 1 and 2 into the body cavity of previously chilled developing pupae of *Platysamia cecropia*. With the Chromoblastomycetes it was difficult to prepare spore suspensions so that mycelial suspensions were used for inoculum. After inoculation, the animals were kept at room temperature and were observed daily for signs of infection.

The results are given in TABLES 1 and 2 and show that only six of the twenty species of *Aspergillus* tested were pathogenic to the insect. Of the other organisms tested, only *Metarrhizium* and the Chromoblastomycetes seemed to be lethal to the animal. In some cases (*e.g.*, *Pleurage* sp.) the fungus seemed to develop for a short time, but died after further incubation. Attempts to culture this organism after it had been in the animal for about a week were

[1] Portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Harvard University.

unsuccessful so it was assumed that the insect was able to destroy the fungus.

It is apparent from the preceding results that infectivity of the *Aspergilli* toward *P. cecropia* does not follow strict taxonomic lines. Members of the *A. flavus-oryzae, A. niger, A. fumigatus, A. tamarii* and *A. flavipes* groups are pathogenic, but the majority of the parasites lie in the *A. flavus-oryzae* group or in the *A. tamarii* group. This latter group, according to Thom and Raper (**1945**), is intermediate between the *A. flavus-oryzae* and *A. wentii*

TABLE 1

RESULTS OF PATHOGENICITY TESTS OF REPRESENTATIVE ASPERGILLI ON *Platysamia cecropia* L.

Group	Organism	Source	Culture number	Results
A. clavatus	*Aspergillus clavatus* Desm	Mrs. E. Gardiner	None	−
A. clavatus	*Aspergillus giganteus* Wehmer	Mrs. E. Gardiner	None	−
A. fumigatus	*Aspergillus fumigatus* Fresenius	QMC Laboratory	PQMD 6b	−
A. fumigatus	*Aspergillus fischeri* Wehmer	QMC Laboratory	Fl All	−
A. glaucus	*Aspergillus ruber* (Brem.)	Harvard	None	−
A. nidulans	*Aspergillus unguis* (Emile-Weil and Gaudin) Thom and Raper	QMC Laboratory	PQMD 30b	−
A. ustus	*Aspergillus ustus* (Bainier) Thom and Church	Harvard	W 56	−
A. versicolor	*Aspergillus versicolor* (Vuill.) Tiraboschi	QMC Laboratory	PQMD 17d	−
A. versicolor	*Aspergillus sydowi* (Bain. and Sart.) Thom and Church	QMC Laboratory	PQMD 26h	−
A. flavipes	*Aspergillus flavipes* (Bain. and Sart.) Thom and Church	QMC Laboratory	PQMD 24a	+
A. flavipes	*Aspergillus flavipes* (Bain. and Sart.) Thom and Church	QMC Laboratory	Fla A-14	−
A. terreus	*Aspergillus terreus* Thom	QMC Laboratory	PQMD 17f	−
A. niger	*Aspergillus niger* van Teighem	Harvard	None	−
A. niger	*Aspergillus luchuensis* Inui	Harvard	None	+
A. wentii	*Aspergillus wentii* Wehmer	QMC Laboratory	PQMD 44a	−
A. flavus-oryzae	*Aspergillus flavus* Link	Harvard	None	+
A. flavus-oryzae	*Aspergillus oryzae* (Ahlburg) Cohn	QMC Laboratory	PQMD 22b	+
A. flavus-oryzae	*Aspergillus parasiticus* Speare	QMC Laboratory	Fla A7	+
A. ochraceous	*Aspergillus ochraceous* Wilhelm	QMC Laboratory	PQMD 26b	−
A. tamarii	*Aspergillus tamarii* Kita	QMC Laboratory	PQMD 51h	+

groups and is unquestionably related to the former. Thus, the *flavus-oryzae* group would seem to be quite readily adapted to the parasitic habit, and the data in TABLE 3, which indicate that seven orders of insects are attacked by these fungi, seem to bear this out.

It is of interest to compare these results with what has been found by other investigators. For example, within the genus *Aspergillus* members of every one of the fourteen groups except the *A. ustus, A. wentii* and *A. candidus* groups have been found to parasitize insects. Blochwitz (**1929**) showed that several diverse groups were pathogenic to *Drosophila*, while representatives of six of them

were listed by Charles (**1941**) as parasitizing bees. Burnside (**1930**) tried to explain the differences in infectivity within the Aspergilli on the basis of the resistance of spores to the action of the acids in the digestive tract of the honeybee. This does not take into consideration pathogens that infect through the surface of the animal nor does it explain why species like *Aspergillus wentii* with relatively thick spore walls (resembling *A. flavus* in this respect) fail to infect the animal. In the case of the pupae of *Platysamia cecropia*, it is not a question of the resistance of the spores to acids in the digestive tract since, in these experiments, the spores were

TABLE 2

RESULTS OF PATHOGENICITY TESTS OF CERTAIN FUNGI ON *Platysamia cecropia* L.

Organism	Source	Culture number	Results
Penicillium chrysogenum Thom	QMC Laboratory	PQMD 51b	−
Penicillium notatum Westling	Harvard	SN 40	−
Paecilomyces variota Bainier	QMC Laboratory	Fla B6	−
Metarrhizium brunneum Petsch	QMC Laboratory	PQMD 191	+
Fonsecaea pedrosoi Brumpt var. typicus	Miss M. Silva	None	+
Fonsecaea pedrosoi Brumpt var. communis	Miss M. Silva	None	+
Alternaria tenuis Nees.	Mr. L. Kaplan	None	−
Pleurage curvula (De Bary) Kuntze	Mr. C. Wilson	None	−
Helminthosporium maydis Nisik. and Miyake	Mr. F. Orillo	None	−
Helminthosporium carbonum Ullstrup	Mr. F. Orillo	None	−

injected directly into the body cavity. Johnston (**1915**) suggested that, within the *A. flavus* group, infectivity was not dependent on morphology but was a function of the strain involved. However, differences in strain must depend on a complex of physiological and morphological characters so that an adequate explanation on such a basis would necessitate the investigation of all these factors.

As for the pathogenicity toward *P. cecropia* of genera more or less closely related to *Aspergillus*, species of *Penicillium* and *Paecilomyces* do not infect the pupae (TABLE 2). This is in keeping with the finding of other workers (Metalnikov and Toumanoff **1928**; Toumanoff **1928**; Burnside **1927**; Boyce and Fawcett **1947**) who found that certain Penicillia were not pathogenic to the insects with which they worked. However, certain species of this genus

parasitize insects (Charles **1941**) so that the animal's resistance to these fungi can not be considered to be absolute.

Of interest among the other fungi which produced fatal infections in the pupae of *P. cecropia* were the two organisms causing Chromoblastomycosis in humans, *Fonsecaea pedrosoi* Brumpt var. *communis* and var. *typicus*. These fungi spread within the host and killed the insect in some cases, although in others the pupa was able to mature and to produce the adult. The results obtained with *Metarrhizium brunneum* Petch were not unexpected since this

TABLE 3

LIST OF INSECT AND ARACHNID HOSTS FOR SPECIES OF THE GENUS *Aspergillus*

Animal attacked	Order	Pathogen	Reference
Tetranychus telarius L.	Acarina (Arachnida)	*A. depauperatus*[1]	Petch 1948
Pseudococcus boninensis Kuw.	Homoptera	*A. parasiticus*	Charles
Pseudococcus gahani Green	Homoptera	*A. parasiticus*	Charles
Pseudococcus sacchari	Homoptera	*A. flavus-oryzae group*	Nattrass
Phenococcus gosypii T. & C.	Homoptera	*A. parasiticus*	Boyce & Fawcett 1928
Palaeococcus rosae R. & H.	Homoptera	*A. flavus*	Charles
Lepidosaphis ulmi	Homoptera	*A. depauperatus*[1]	Thom and Raper
Aspidiotus sp.	Homoptera	*A. depauperatus*[1]	Thom and Raper
Mordellistena sp.	Orthoptera	*A. flavus*	Charles
Apion occidentale Fall	Orthoptera	*A. flavus*	Charles
Schistocerca gregaria Forsk.	Orthoptera	*A. flavus*	Lepesma
Schistocerca camellata	Orthoptera	*A. parasiticus*	Charles
Dichrophis arrogans	Orthoptera	*A. flavus*	Charles
Dichrophis elongatus	Orthoptera	*A. flavus*	Charles
Malacosoma americana F.	Lepidoptera	*A. flavescens*	Gee and Massey
Prodenia sp.	Lepidoptera	*A. niger*	Charles
Pyrausta nubilalis Hbn.	Lepidoptera	*A. niger, A. flavus, A. parasiticus*	Met., Toum., Toum.
Bombyx mori L.	Lepidoptera	*A. flavus*	Nomura from Blochwitz
Diatraea saccharalis F.	Lepidoptera	*A. parasiticus*	Charles
Harmolita sp.	Hymenoptera	*Aspergillus* sp.	Charles
Apis mellifera L.	Hymenoptera	*A. flavus, A. fumigatus, A. nidulans, A. oryzae, A. parasiticus, A. glaucus, A. ochraceous, A. effusus*[2]	Burnside, Howard, Turesson, Cury, Charles
Microbracon hebetor Say	Hymenoptera	*A. parasiticus*	Charles
Atta singularis Guer.	Hymenoptera	*A. tamarii*	Charles
Culex sp.	Diptera	*A. niger, A. glaucus*	Galli-Valerio
Anopheles sp.	Diptera	*A. niger, A. glaucus*	Galli-Valerio
Drosophila sp.	Diptera	*A. clavatus, A. niveus, A. fumigatus, A. flavus, A. malignus,*[3] *A. nidulans, A. giganteus, A. galeritus*[4]	Blochwitz
Anastrepha serptulis Wied.	Diptera	*A. sydowi*	Charles
Unidentified termites	Isoptera	*A. flavus*	Boedijn (Blochwitz)
Thermobia domestica (Packard)	Thysanura	*A. flavus*	Previously unpubl.[5]

[1] This organism is placed in the *A. restrictus* series by Thom and Raper (1945)
[2] Belongs to the *flavus-oryzae* group.
[3] Belongs to the *A. fumigatus* group.
[4] Synonym of *A. terreus*.
[5] The fungus was isolated from infected eggs reared by Dr. Charles Remington, who found that the fungus attacked these and prevented further development.

TABLE 4

SUMMARY OF THE RESULTS OF EXPERIMENTS ON THE HOST RANGE OF *Aspergillus flavus* Link

Insect used	Order	Stage tested	Method of inf.	Result
Actias luna L.	Lepidoptera	pupa	injection	+
Telea polyphemus Cram.	Lepidoptera	pupa	injection	+
Callosamia promethea Dru.	Lepidoptera	pupa	injection	+
Haemorrhagia gordius Cram.	Lepidoptera	pupa	injection	+
Epargyreus tityrus Fabr.	Lepidoptera	larva	spray	+
Teneid moth (unident.)	Lepidoptera	larva	spray	+
Popillio japonica Newn.	Coleoptera	adult	spray	−
Coccinelid beetle (unident.)	Coleoptera	adult and pupa	spray	−

fungus, and a related species (*M. anisopliae*), are pathogenic to numerous insects. Several representatives of the Moniliales and a species of *Pleurage* were not pathogenic.

HOST RANGE

The animals used in the following experiments were either collected in the vicinity of the Gray Herbarium in Cambridge, Massachusetts, or were sent by collectors [2] or dealers who generously cooperated by forwarding some of the material.

Essentially the same techniques were used in these experiments as were described in the first paper of this series (Sussman **1950**). Larvae and adult insects were inoculated by spraying a sodium oleate-gelatin suspension of spores (Anderson and Henry **1946**), while controls were sprayed with only the sterile solution. On the other hand, pupae were injected with 0.05 ml. of a distilled water suspension of spores and sterile distilled water injections were used as the control treatment.

The results, as shown in TABLE 4, were considered positive only when 100 per cent infection occurred in treated animals and when none of the controls were affected by the treatment. At least 20 animals were used in each experiment. The fungus attacked all of the Lepidopterans used in these experiments, although the beetles were not infected.

Symptoms similar to those exhibited by *P. cecropia*, when infected with *A. flavus*, appeared in the animals which were sus-

[2] I should like to express my appreciation to Dr. Charles Remington of Yale University and to Mr. Sidney Hessel of Woodmere, New York, who helped me to collect and identify some of the insects with which I worked.

ceptible to the disease. Larvae became sluggish and spasmodic in their movements, discharged fluid from the mouth and anus, and gradually became black in color. Pupae also blackened, and lost the ability to respond to stimuli. In general, the time needed for the infection to manifest itself was about 48 to 72 hours at room temperature.

GENERAL CONCLUSIONS

These results attest once more to the vigor and adaptability of the Aspergilli. Their wide distribution, ability to grow on varied substrates and resistance to environmental changes make it possible for them to grow saprophytically in widely differing habitats. Consequently, many of the reports of insect infections by these fungi do not make clear by experimental means that the fungus is a pathogen rather than a saprophyte upon the dead animal. Moreover, infection in the laboratory, using drastic means of inoculation like injection, does not necessarily prove that such severe infections of insects occur in nature. However, sufficient evidence does exist to prove that these omnipresent fungi, under certain conditions, do adopt the pathogenic habit rather than the saprophytic. It is hoped that further work will elucidate the factors which induce or allow this transformation of habit to occur.

The author would like to express his deepest appreciation to Dr. William H. Weston, under whose guidance this work was done, and to Dr. Carroll M. Williams, who provided materials and help in performing some of these experiments.

SUMMARY

1. Six of twenty representative species of Aspergilli were found to be capable of producing a lethal infection in *Platysamia cecropia* L.

2. Of eight other genera tried, only *Mettarhizium* and *Fonsecaea* proved to be infective to the insect.

3. The host range of *Aspergillus flavus* Link has been extended to include six more genera of the Lepidoptera; the attempt to infect two beetles proved unsuccessful.

DEPARTMENT OF BOTANY,
UNIVERSITY OF MICHIGAN,
ANN ARBOR, MICHIGAN

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AN UNDESCRIBED SPECIES OF PHYSODERMA ON AESCHYNOMENE INDICA

M. J. Thirumalachar and Marvin D. Whitehead

(with 17 figures)

Aeschynomene indica L., popularly known as the pith plant, is a partially submerged aquatic plant rooting in the soil beneath. In the neighborhood of Bangalore, and Banaras, India, large galls incited by a fungus on the submerged parts of the plants were collected. The galls were quite distinct from the nodular intumescences produced naturally on the submerged portions of the stems. A morphological study of the inciting fungus revealed that it is a species of *Physoderma* but showing an unusual type of germination of the resting sporangia in the Bangalore collection. An account of these studies is presented here.

The galls incited by the fungus were mostly on the stems and rarely on the rachis of the compound leaf (Fig. 1). They were usually large, cushion-shaped, measuring up to 20 mm. long and 10 mm. broad. Young galls resembled small tubercles on the stems and were pale yellowish-brown in color. When the mature galls were cut in cross section, a chocolate-brown mass of spores escaped.

For cytological studies, the material was fixed in Allen's modification of Bouin's fluid, and microtome sections of 8 to 10 μ thickness were cut and stained with Heidenhain's iron-alum haematoxylin with eosin B. as a counterstain. Structures of the rhizomycelium, turbinate organs and other details were observed in lactophenol mounts stained with acid fuchsin or cotton blue. For studying the germination stages, resting sporangia were teased out of mature galls and germinated on slides by the method suggested by Thirumalachar (2).

Sections through the galls indicated that the fungus was entirely intramatrical, developing tenuous intracellular rhizomycelium and numerous resting sporangia filling up the host cells (Fig. 2).

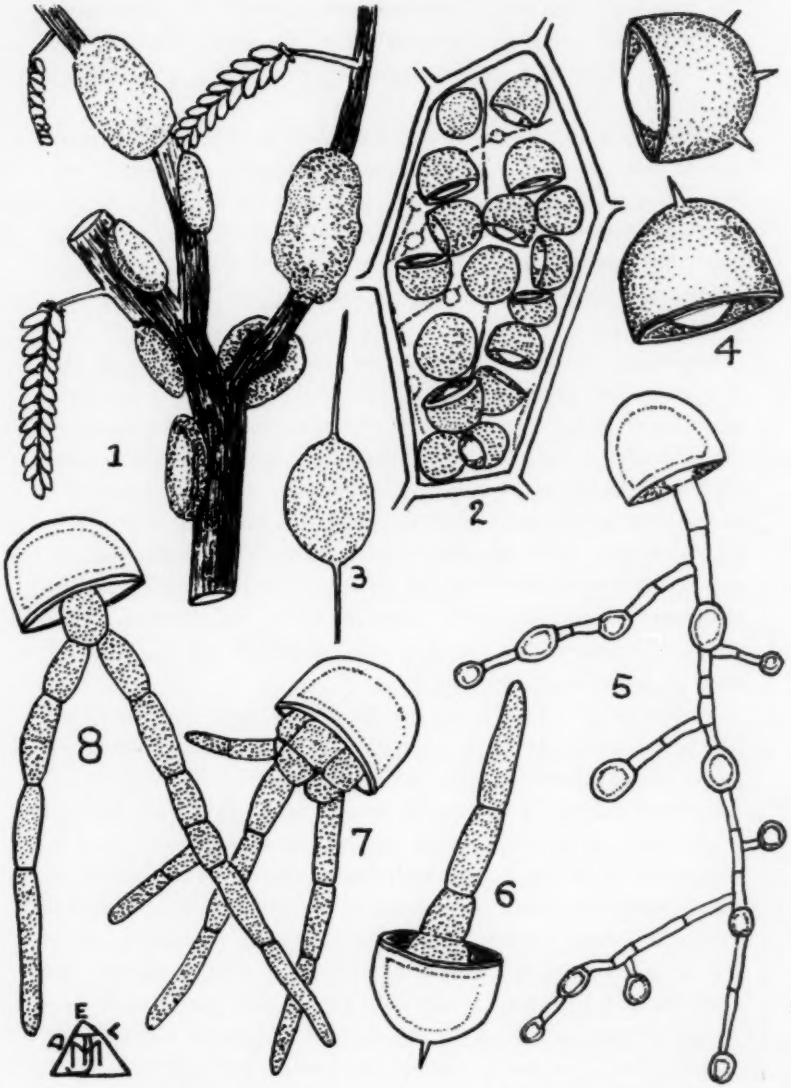
The rhizomycelium was branched, and showed the intercalary spindle-shaped swellings characteristic of the genus *Physoderma* (FIG. 3). These rhizomycelia traversed from cell to cell, bearing numerous resting sporangia.

Mature resting sporangia were distinctly asymmetric, flattened on one side (FIG. 4). Young sporangia usually showed one to three acicular haustorial processes which were connected with the inner cytoplasm. In mature sporangia these may or may not occur since they break away. Mature resting sporangia were reddish-brown, hemispherical, flattened on one side, 25–28 μ broad and 19–25 μ high.

Histological studies indicated that the fungus stimulated the division of the parenchymatous cells of the host. The divisions were mostly periclinal, resulting in the formation of radiating rows of thin-walled rectangular cells. The enlarged infected cells became filled with the resting sporangia. While there were no locules produced as in some species of *Physoderma*, the grouping of the resting sporangia often took place in irregular patches, so that the gall was not uniformly filled with the spores. Examination of the galls of infected plants taken from ponds after they had dried up in the summer indicated that the sori were converted into a powdery mass of chocolate-brown spores.

Germinations of the resting sporangia were made on glass slides. The germination studies of material collected at Banaras and Bangalore indicated entirely different types of developmental pictures. The same fungus was involved in both the cases as the morphology of the sori and sporangia of the two collections was identical. As will be described later, the germination behavior of the spores in the Bangalore material was so unlike that of *Physoderma* that it was at one time considered feasible to allocate a separate genus for its accommodation. Later germination studies carried out from Banaras specimens revealed the typical germination of *Physoderma*, so that we are at present inclined to consider the mode of germination in the Bangalore specimen as an anomalous type.

The resting sporangia of the Banaras collection germinated after four days incubation at room temperature (20–24° C.). The first sign of germination was the lifting up of the saucer-shaped lid by the protrusion of the conical prolongation of the endospore. In

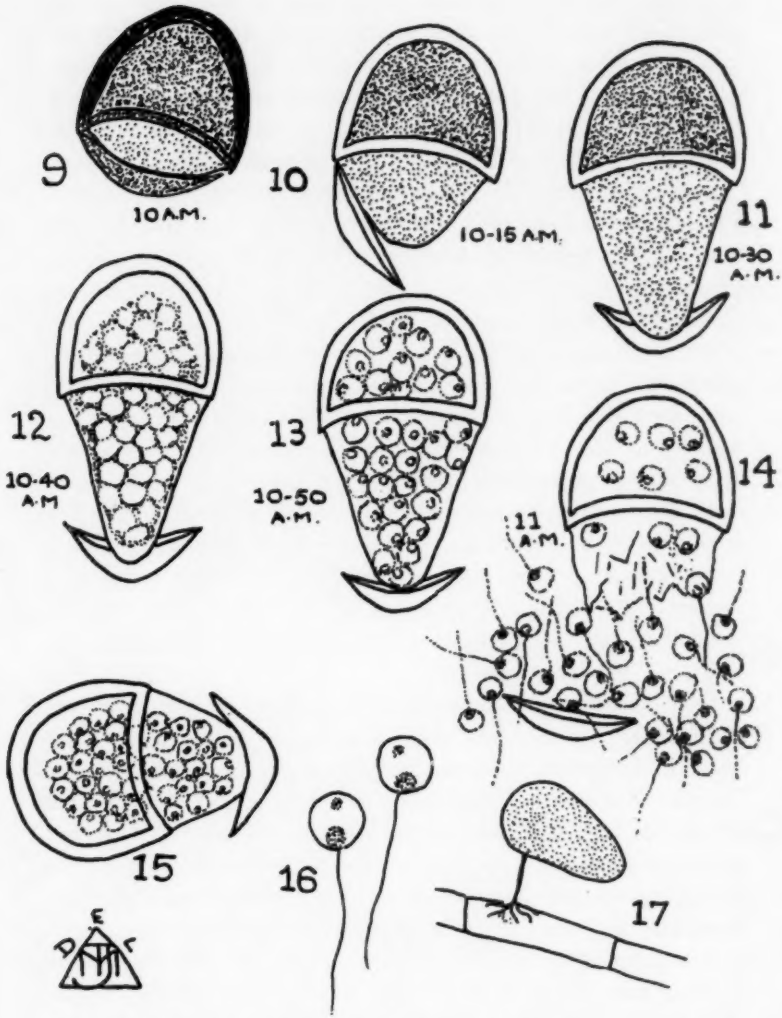


FIGS. 1–8. Infected shoot showing the galls, nat. size. 2. Rhizomycelium and resting sporangia within the host cell, × 400. 3. Spindle-shaped intercalary swelling, × 1800. 4. Mature resting sporangia, × 750. 5–8. Germination stages of the Bangalore specimens showing the septate germ tubes and in figure 8 chlamydospore formation, × 750.

some cases the lid was pushed aside, while in other cases the lid was carried over at the apex of the conical prolongation of the endospore. The entire germination process was completed within the duration of one hour and the various stages were followed (FIGS. 9–14). After the prolongation of the endospore (FIGS. 10, 11), the granular contents of the resting sporangia showed fragmentation (FIG. 12) and gradually rounded off into zoospores. The zoospores at the beginning were closely appressed to each other and later separated filling up the space within the sporangium (FIGS. 13, 15). There was no swarming movement of the zoospores within the sporangium. There was no visible deliquescence of the apical papilla of the sporangium before the discharge of the zoospores, but on the other hand the sporangium ruptured with some force scattering the zoospores and the operculum some distance (FIG. 14). The zoospores after being discharged remained quiescent for about three minutes, then gradually started movement, later swimming away with rapid darting movements. The zoospores were globoid to pyriform, 5–7 μ in diameter, with a large excentric hyaline globule on the posterior side and a tiny refractive globule in the center (FIG. 16). In stained preparations, the single posterior cilium of each zoospore was distinct and measured up to 20 μ in length.

Slide cultures of the zoospores were incubated in moist chambers continuously for the study of their behavior. Observations after 48 hours indicated the development of ephemeral sporangia of the fungus. The ephemeral sporangia were slipper-shaped, attenuated slightly at one end and attached to the substrate by branched rhizoidal processes (FIG. 17). The development of zoospores within the ephemeral sporangia was not traced, though the fragmentation and rounding of their contents was observed.

The germination behavior of the spores in the material collected at Bangalore was as follows. The first sign of germination was the rupture of the saucer-shaped lid situated on the flattened side and the emergence of a stout germ tube (FIG. 6). There was no evidence of the pushing up of the operculum. The germ tube rapidly elongated becoming closely septate. The cells appeared somewhat moniliform due to the constrictions at the septa. As development proceeded, several branches were formed (FIG. 8).



FIGS. 9–17. Stages in the germination of a single resting sporangium in the collections made at Banaras, showing the dehiscence of the operculum, formation of zoospores and their dispersal, × 1000. 15. Same stage as figure 13, × 1000 16. Zoospores showing cilia and excentric hyaline bodies, × 1800. 17. Epibiotic ephemeral sporangium, × 2000.

In some cases the protruding germ tube, instead of rapidly elongating, with the formation of transverse septa, produced a group of cells by anticlinal and periclinal cell division and from these protrudances germ tubes developed (FIG. 7). Several of the slides with the germinating spores were incubated for prolonged periods in a moist chamber at room temperature. In this case, the germ tubes gradually ramified, becoming thin and tenuous, with sparse cell contents. Several of the cells in the germ tube became filled with rich cell contents and rounded off into chlamydospore-like bodies that were both terminal and intercalary in position (FIG. 5).

The germination of resting spores described above for the specimen collected in Bangalore indicates a condition unknown in any of the species of *Physoderma* or other chytrids. The method of chlamydospore formation in the germ tube is similar to the chlamydospore formation within the host tissue in species of *Protomyces*. Discovery of more forms showing these intermediary characters may bring *Physoderma* and *Protomyces* closer than they are believed to be at present. For determining the identity of the present fungus, germination by zoospores is taken as representing the normal type of germination and the fungus is placed under *Physoderma* as an undescribed species. No species has been previously described on this host genus (**1**).

Physoderma Aeschynomenis Thirumalachar & Whitehead sp. nov.[1]

Rhizomycelio endobiotico tenui, delicatulo, ramoso, inflationibus fusoideis intercalaribus praedito: sporangiis fugacibus epibioticis, soleaeformibus, processibus ramosis rhizoidalibus affixis; sporangiis perdurantibus cellulas hospitis implentibus, endobioticis, rufo-brunneis, hemisphaericis, in latere uno applanatis et operculo patelliformi ad depressionem praeditis, in latere convexo 1–3 processibus acicularibus haustorialibus ornatis, 25–28 × 19–25 μ; sporangiis perdurantibus operculo dehiscentibus et endosporis protrusis germinatibus; zoosporis 32–48, 5–7 μ in diam., cilio usque 20 μ longo praeditis.

Hab. in caulibus rachidibusque *Aeschynomenis indicae*, India.

Rhizomycelium endobiotic, tenuous, delicate, branched, with intercalary spindle-shaped swellings. Ephemeral sporangia epibiotic, slipper-shaped swellings, attached by branched rhizoidal

[1] The authors are indebted to Dr. Edith K. Cash, associate mycologist, Division of Mycology and Disease Survey, U.S.D.A., for the preparation of the Latin diagnosis.

processes. Resting sporangia endobiotic, filling the host cells, reddish-brown, hemispherical, flattened on one side, with saucer-shaped lid on the depression, with one to three acicular haustorial processes on the convex side, measuring 25–28 × 19–25 μ; resting sporangia germinating by the dehiscence of the lid, and protrusion of the endospore; zoospores 32–48 in number, 5–7 μ in diameter, with a long cilium up to 20 μ.

Hab. on the stems and rachis of *Aeschynomene indica* L., Banaras Hindu University, leg. M. J. Thirumalachar, 12–11–1949 (Type) leg. M. S. Pavgi, Oct. 1950.

Portions of the type specimen collected at Banaras, India, are deposited in the Mycological herbarium of the U.S.D.A., Bureau of Plant Industry, Beltsville Maryland; Herb. Crypt. Ind. Orient, New Delhi, India; Commonwealth Mycological Institute, Kew, England.

BANGALORE, INDIA,
AND
DEPARTMENT OF PLANT PHYSIOLOGY AND PATHOLOGY,
TEXAS AGRICULTURAL EXPERIMENT STATION,
COLLEGE STATION, TEXAS

LITERATURE CITED

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2. **Thirumalachar, M. J.** A method for germinating and staining teleutospores. Jour. Indian Bot. Soc. **19**: 70–75. 1940.

NOTES ON CORYNELIA OREOPHILA (SPEG.) STARB. AND CLOSELY RELATED SPECIES

HARRY MORTON FITZPATRICK [1]

(WITH 11 FIGURES)

In the writer's monographic treatment of the Coryneliaceae,[2] published in 1920, nine species were recognized in the genus *Corynelia*. Twenty-two years later, in his Revisionary Studies in the Coryneliaceae,[3] two of these, *C. fructicola* and *C. bispora*, were removed from the genus and made the types of two new genera, *Corynecliospora* and *Lagenulopsis*, respectively. The seven remaining species constitute the genus *Corynelia* as we know it to-day. Thus emended, *Corynelia* differs strikingly from the other genera of the family in that the ascocarp at maturity is deeply and widely cleft, not merely apically perforate. All the species embraced in *Corynelia* are parasitic on *Podocarpus*. Four of them, *C. oreophila*, *C. brasiliensis*, *C. portoricensis*, and *C. jamaicensis*, are known only from the Western Hemisphere, and clearly are more closely related to each other than to the other members of the genus. It is with these four species that the present paper is concerned.

In these species the upper portion of the ascocarp is definitely lobed, with prominent grooves lying between the lobes and extending far down the sides. Dehiscence occurs along the entire length of the grooves, the lobes then pull apart, and the ascocarp opens widely exposing the inner surface of its wall. In two of the species, *C. oreophila* and *C. brasiliensis*, known only from continental South and Central America, the ascus is typically 8-spored, with

[1] This posthumous paper, unfinished at the time of the death of Dr. Fitzpatrick, was completed and submitted for publication by Julian H. Miller. See footnote 4.

[2] Mycologia **12**: 206–267. Figures 1–49. 1920.

[3] Mycologia **34**: 464–514. Figures 1–35. 1942.

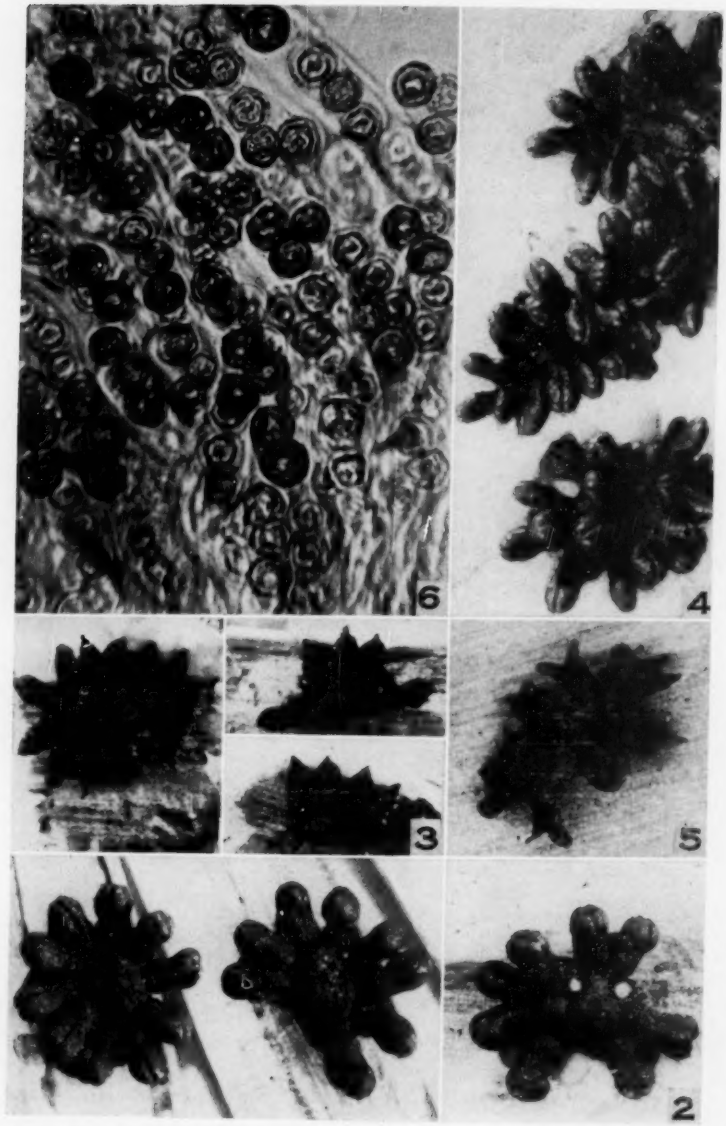
fewer-spored asci so unusual as to be easily overlooked. The other two species, *C. portoricensis* and *C. jamaicensis,* found as yet only in the West Indies, have the ascus typically few-spored, an ascus with eight fully formed normal spores not yet having been encountered. The upper portion of the ascocarp in *C. oreophila* and *C. jamaicensis* is usually trilobed, while that of *C. portoricensis* and *C. brasiliensis* is usually bilobed. Variation in the number of lobes of the ascocarp occurs in all four species, and the discovery of borderline material between *C. oreophila* and *C. brasiliensis* or between *C. portoricensis* and *C. jamaicensis* has been regarded by the writer as a possibility. As yet, however, no collection has been examined in which it is sufficient to cause confusion concerning specific limits. The pairs of species which differ in the number of ascospores in the ascus are clearly and widely separated by this one character alone.

The four species evidently arose from a common ancestry in which the ascocarp was probably bilobed and the ascus 8-spored, and underwent gradual divergence as the geographic range altered. A chart prepared in amplification of this hypothesis was published in the writer's earlier monographic treatment of the genus. Of these four species, *C. oreophila* was first described. The three with geographic specific epithets were erected simultaneously by the writer in 1920, and were based on a limited amount of material from the indicated regions. Except for the fact that *C. jamaicensis* is now known from Cuba, the three names are still wholly appropriate. The various collections of *C. portoricensis* seen by the writer are all from the type locality, and the known material of *C. brasiliensis,* though abundant, is all from São Paulo. In contrast, collections of *C. oreophila* have been made in Costa Rica, Colombia, Venezuela, Bolivia, Brazil, Chili, and Argentina.

The species of the host genus *Podocarpus* occur chiefly in the Southern Hemisphere. In the tropics they are found usually at high altitudes, often in inaccessible places. There are relatively few mycologists in these regions, and though the writer has urged that additional collections of *Corynelia* be made the years have brought little response. Our knowledge of the geographic distribution and morphological variation of the species is consequently less than is to be desired. From time to time materials are re-

FIG. 1. *Corynelia brasiliensis* on *Podocarpus Lambertii*. 1. Scattered stromata bearing ascocarps; stromata erumpent on both leaf surfaces and the young green portion of the branchlet. × 1.

ceived from correspondents or encountered in herbaria. A few specimens of unusual interest have been received in recent years. The present paper deals with several of these, chiefly with two collections of *C. oreophila* from Venezuela which are atypical, as com-



FIGS. 2, 3. *Corynelia brasiliensis.* 2. Three stromata; the central cushion-like portion of each bordered by a row of mature, as yet unopened, chiefly bilobed ascocarps, × 11. 3. Three stromata bearing aged ascocarps which,

pared with all earlier known materials, and necessitate alteration in the revised specific diagnosis as published by us in 1942. In it the following words occur: "ascocarp typically trilobed and trisulcate: occurrence of bilobed or quadrilobed individuals rare; ascocarps closed or wedge-shaped in age, as in *C. brasiliensis,* not observed."[4] One of the *C. oreophila* collections of atypical material recently received from Venezuela (Myc. Explor. Ven. *4072* or C.U. *31702*) is interesting in that it is the first material of this species seen in which ascocarps with bilobed apices are numerous among the trilobed ones. Figure 8 shows a stroma bearing both trilobed and bilobed ascocarps. Counts in this collection were made of ascocarps on eight stromata and 32 per cent were bilobed, 68 per cent trilobed and no quadrilobed ascocarps were found. The bilobed tips were of the shape of those of *C. portoricensis* (FIG. 4). If instead they were of the shape of those of *C. brasiliensis* (FIGS. 2–3) the separation of *C. oreophila* and *C. brasiliensis* would become more difficult.

No aged material is included in this collection, but wedge-shaped apices in age would be unlikely on account of the fact that the broad side of the lobe does not parallel the apical cleft.

The asci in this collection were chiefly 8-spored (FIG. 9). Counts of 119 asci gave 90 per cent with 8, 6 per cent with 7, 1 per cent with 6, 2 per cent with 5, and 1 per cent with 4.

The second Venezuelan collection (Inst. Trop. Agri. *5046* or C.U. *31747*) has special interest in that it includes numerous quadrilobed individuals among the trilobed ones (FIG. 10). In counts of 107 stromata, 89 per cent of the ascocarps were trilobed, 10 per cent quadrilobed, and 1 per cent bilobed. This then is quite dif-

[4] Statement of J. H. Miller: The paper up to this point was written by Dr. Fitzpatrick, and I have prepared the rest from notes that he left. He also had completed and mounted the photomicrographs.

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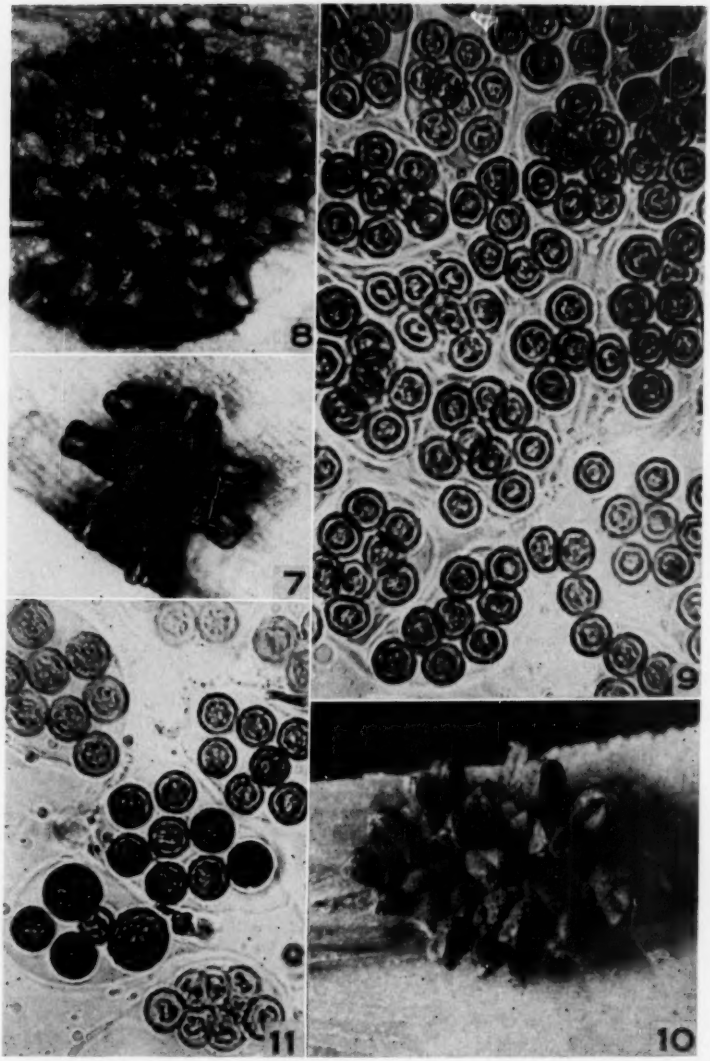
after opening and disseminating their spores, have closed to the wedge shape typical of aged specimens in this species, × 11.

FIGS. 4–6. *C. portoricensis* (C.U. *31744* or I.T.A. *7244*). 4. Stromata bearing mature, as yet unopened ascocarps, chiefly bilobed in this species, × 11. 5. Mature ascocarps showing dehisence by transverse cleft along the line of the terminal groove; the ascocarp tending to remain open, not closing to wedge-shaped, × 11. 6. Asci, chiefly three-spored, × 510.



FIGS. 7–11. *Corynelia orcophila*. 7. Stroma bearing a single encircling row of the trilobed ascocarps typical of this species, × 11. 8. Stroma showing both trilobed and bilobed ascocarps; the latter resembling in form those of *C. portoricensis* rather than those of *C. brasiliensis;* Venezuela material collected by Barnes (M.E.V. *4072* or C.U. *31702*), × 11. 9. Asci, from same

ferent from the collection described above. Here we have two collections differing widely in the lobing of the ascocarps.

Counts were made of numbers of ascospores per ascus in ascocarps with four lobes in this collection. In 261 asci there were 93 per cent with 8 ascospores, 3 per cent with 7, 2 per cent with 6, 1 per cent with 5 and 1 per cent with 3. Figure 11 shows typical 8-spored asci and one with 5 spores.

In addition to the above there is a recent collection of *C. portoricensis* (Inst. Trop. Agri. *7244* or C.U. *31744*) which contains material of aged ascocarps standing wide open and empty with the lobes spread wide apart (FIGS. 4–5). Not in a single instance is any evidence given of a tendency for the lobes to close together in age to form a definitely wedge-shaped apex such as characterizes material in *C. brasiliensis* (FIG. 3).

In another recent collection from the same Puerto Rican locality (C.U. *31764*) 603 ascocarps were counted and 96.2 per cent were bilobed, and 3.8 per cent trilobed. In another count of this material out of a total of 347 ascocarps 11.8 per cent were closed, 10.9 per cent wedge-shaped, and 77.3 per cent open. The wedge-shaped ones were found only on one leaf, and all others after dehiscence remained open. The wedges are not identical with those of *C. brasiliensis*.

Asci from this collection from C.U. slide No. *9655* show from a count of 250, 3.2 per cent with 1 ascospore, 9.2 per cent with 2, 86.4 per cent with 3 (FIG. 6) and 1.2 per cent with 4.

In conclusion the description of *C. oreophila* in the second paper [3] should read instead of "bilobed or quadrilobed individuals rare," both types are of common occurrence but trilobing predominates. The species *C. oreophila* and *C. brasiliensis* are still united by having chiefly 8 spores to the ascus, and can be separated on the wedge-shaped ascocarp apex in the latter and not in the former.

collection, chiefly eight-spored, × 510. 10. Stroma bearing both trilobed and quadrilobed ascocarps; apex of one of the latter shown clearly at the center of the group; Venezuela material collected by Tamayo (I.T.A. *5046* or C.U. *31747*), × 11. 11. Asci from same collection, chiefly eight-spored, but a single five-spored ascus included to illustrate variation in spore size in fewer-spored asci, × 510.

C. portoricensis now has been shown to have some wedge-shaped apices as in *C. brasiliensis*, but can be distinguished on few ascospores, chiefly 3, to the ascus instead of 8; and the very large per cent of bilobed ascocarps even in the new material still affords a separation from *C. jamaicensis*.

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY,
ITHACA, NEW YORK

NEW SPECIES OF THE PERONOSPORACEAE [1]

CHARLES GARDNER SHAW [2]

(WITH 8 FIGURES)

Taxonomic studies of the Peronosporaceae during the past several years have brought to light five species apparently not described previously. The first two species discussed were included in an unpublished thesis submitted to the Graduate School of the University of Wisconsin (**9**). The investigation of the latter three species was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure No. 171.

Peronospora Davisii sp. nov.

Caespitulis gracillimis, griseo-albis, tergum foliorum nonnulla parte subtegentibus. Conidiophoris plerumque singulis e stomatibus exeuntibus, non septatis, albis, 200–480 μ altis, trunco 2/3 totius altitudinis efficiente, 7–11 μ crasso, ramis (3–)4–5(–6)ies subdichotome ramosis, flexuosis vel curvatis; furcis terminalibus 3–19 μ longis, subacutis, curvatis, inaequalibus, ramo uno ramum praecedentem continuente, incurvato, ramo altero breviore, retroflexo. Conidiis ellipsoideis, brunneis, 26–42 μ longis, 16–25 μ latis. Longitudine media 34.2 μ; latitudine media 20.6 μ. Oosporis ignotis. (FIG. 6.)

Hab. in foliis vivis *Dracocephali parviflora* Nutt.

Foliicolous; infected area somewhat angular, limited by the veins, tan, becoming cinnamon-brown above; yellowish below, then brown, mat very sparse, often inconspicuous, cinereous; conidiophores hypophyllous, aseptate, hyaline or lightly tinged, 1–2 emerging from a stoma, (3–)4–5(–6) times monopodially to subdichotomously branched; 200–480 × 7–11 μ; crown about $\frac{1}{3}$ the total height, open, main branches flexuous to curved; ultimate branches subacute, variously inserted, unequal, curved, 3–19 μ, the axial longer, incurved, the abaxial recurved; conidia at first hyaline, then dark

[1] Scientific Paper No. 993, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, The State College of Washington, Pullman.

[2] Associate Professor and Associate Pathologist, Division of Plant Pathology, The State College of Washington.

brown, ellipsoid, ends somewhat bluntly pointed, very large, 26–42 × 16–25 μ (Mean 34.2 × 20.6 μ); oospores not observed. (FIG. 6.)

On Labiatae: conidia on *Dracocephalum parviflorum* Nutt.

Type in the herbarium of the University of Wisconsin, Madison, Wisconsin. Collected at Gordon, Douglas Co., Wisconsin, July 17, 1907, by J. J. Davis.

Davis (**3**) first listed this species as *Per. Lophanthi* Farlow, adding, however, a correction slip reading: "This should not be referred to *Peronospora Lophanthi* Farl. It differs in the larger and more loosely branched conidiophores and the oval conidia 30–36 × 20 microns." In his provisional list (**4**) *D. parviflorum* is listed as a host for *Per. Hedeomatis* Kell. & Sw.

Three specimens, collected at the type locality, were available for study. The differences pointed out by Davis (**3**) as distinguishing the new species from *Per. Lophanthi* serve equally well to separate it from *Per. Hedeomatis*. Of the species discussed by Gäumann (**7**), *Per. rossica* Gaum. on *D. thymiflorum* L. from Russia has smaller, more nearly subspherical conidia (average 23.90 × 18.59 μ) while *Per. stigmaticola* Raunkaier, occurring on *Mentha* spp. in northern Europe, has very narrow, elliptical conidia for which Gäumann (**7**) gives the following mean measurements: 34.40 × 13.28 μ. In spite of the few specimens available, the differences between the conidia from *D. parviflorum* and those from other members of the Labiatae are considered beyond the limits of normal variability. A careful search failed to reveal the oospores. Germinated conidia were not observed, but there was no indication of an apical pore on the ungerminated conidia.

Conidial measurements for the three downy mildews occurring on species of the Labiatae in Wisconsin are given below. One hundred conidia from each of three different specimens were measured for each of the three species.

Host	Range (μ)	Mean (μ)
Per. Lophanthi Farl. on *Agastache scrophulariaefolia* (Willd.) Ktze.	11–19 × 13–25	15.0 × 19.2
Per. Hedeomatis Kell. & Sw. on *Hedeoma hispida* Pursh.	12–20 × 18–30	15.4 × 23.9
Per Davisii on *Dracocephalum parviflorum* Nutt.	16–25 × 26–42	20.6 × 34.2

Plasmopara Myosotidis sp. nov.

Caespitulis gracillimis, difficiliter visibilibus, tergum foliorum nonnulla parte subtegentibus. Conidiophoris hypophyllis, sparsis, hyalinis, septatis, 160–380 μ altis, trunco ½ totius altitudinis efficiente, 7–10 μ crasso, basi leviter tumida (13 μ), singulis vel binis e stomatibus exeuntibus, 3–5ies pinnatis; 3–6 ramis primariis, ramis angulo recto insertis, ramis subultimis in ramulos 2–4 divaricatos exeuntibus; ramulis ultimis rectis, leviter conicis, 5–11 μ longis, apice truncatis. Conidiis oblongo-ellipsoideis, hyalinis, apice poris munitis, basi pedicellatis, 20–23 × 14–16 μ. Oosporis ignotis. (FIG. 8.)

Hab. in foliis *Myosotidis laxae* Lehm.

Foliicolous; infected area irregular, at first chlorotic above, becoming brown; appearing unoccupied below; conidiophores hypophyllous, hyaline, with plug-like septations, emerging from the stomata singly or in 2's, 3–5 times monopodially branched, 160–380 × 7–10 μ, occasionally basally swollen to 13 μ; crown ½ the total height, primary branches 3–6 in number, arising at right angles, straight; ultimate branches 2–4 in number, slightly conic, straight, truncate, 5–11 μ long; conidia hyaline, oblong-ellipsoid, indistinctly poroid apically, basally pedicellate, 20–23 × 14–16 μ; oospores not seen. (FIG. 8.)

On Boraginaceae: conidia on *Myosotis laxa* Lehm.

Type in the herbarium of the University of Wisconsin, Madison, Wisconsin. Collected July 20, 1922, at Arena, Iowa County, Wisconsin, by J. J. Davis.

Davis (**5**) stated: "*Peronospora myosotidis* DBy. A very scanty collection on upper leaves of *Myosotis laxa* in July at Arena is supposed to be of this species, but the material was not sufficient for proper examination." The phanerogamic herbarium at the University of Wisconsin contains a specimen of *Myosotis laxa* Lehm. bearing the same collection data as does the fungus collection. The fungus specimen consists of four small leaves, which agree with the upper leaves of *Myosotis laxa*. There seems to be no reason for questioning the host determination. Davis certainly did not examine the fungus under the compound microscope. As is evident from the description above and figure 8, this material cannot be referred to *Peronospora Myosotidis* de Bary. Apical pores ("papillae" of other authors) are present on the conidia; the pedicillate nature of the conidia is readily apparent. The conidiophores are those of a typical *Plasmopara:* the branching is at right angles; septations are present in the trunk; the ultimate branches occur in groups of 2–4 and are equal in length.

While several species of *Peronospora* occur on various members of the Boraginaceae, evidently no species of *Plasmopara* has been described previously.

Peronospora Jacksonii sp. nov.

Maculis indefinitis et irregularibus, fusco-cinereis supra, infra pallido-viridibus tantum, 2–3 cm. latis; caespitulis griseo-albis, floccosis. Conidiophoris hypophyllis, non septatis, hyalinis, fasciculatis, e stomatibus exeuntibus, 310–410 μ altis, trunco ½ vel minus totius altitudinis efficiente, 8.5–10 μ crasso, raro basi leviter tumida; (4–)6–7ies subdichotome ramosis, ramis primariis rectis vel subrectis et angulo acuto insertis, ramis minoribus curvatis, cacumen densum efficientibus; furcis terminalibus 2.5–25 μ longis, leviter curvatis, inaequalibus, subacutis, ramo uno ramum praecedentum continuente, leviter incurvato, ramo altero breviore, leviter retroflexo. Conidiis pallide fuscis, ellipsoideis, ad extremos curvatis, 18–30 × 11–17 μ, plerumque 20–25 × 13–15 μ; oogoniis variae formae, subglobosis vel late ellipsoideis, 55–88 × 40–70 μ, membrana tenui, hyalina; oosporis globosis, 29–52 μ diam., laxis; exosporio tuberculato, primo pallide flavo, tum flavo, denique fulvo, 4–7 μ crasso, endosporio hyalino, difficiliter per exosporium visibili, 4–5 μ crasso. (FIGS. 1, 4.)

Hab. in foliis *Mimuli guttati* DC.

Foliicolous; infected area indefinite and irregular, 2–3 cm. or more across, fuscous-cinereous above, merely chlorotic and covered with a conspicuous greyish-white floccose mat below; conidiophores hypophyllous, aseptate, hyaline, fasciculate from the stomata, (4–)6–7 times monopodially branched, 310–410 × 8.5–10 μ, base rarely bulbous, and then indistinctly so, trunk ½ or less the total height, primary branches straight or nearly so and inserted at acute angles, smaller branches somewhat curved and twisted, forming a dense intertwined crown; ultimate branches inserted at acute to right angles, somewhat curved, unequal, acute, 2.5–25 μ long, the axial the longer, and slightly incurved, the abaxial somewhat recurved; conidia light brown, ellipsoid, ends rounded, 18–30 × 11–17 μ, mostly 20–25 × 13–15 μ; oogonia variable in shape, irregularly subspherical to broadly ellipsoid, 55–88 × 40–70 μ, wall thin, hyaline; oospores spherical, 29–52 μ in diam., free in the oogonia, exospore irregularly tuberculate, at first light yellow, becoming golden yellow and finally yellowish brown, 4–7 μ thick, endospore 4–5 μ thick, hyaline, difficult to discern through the exospore. (FIGS. 1, 4).

On Scrophulariaceae: conidia and oospores on *Mimulus guttatus* D. C.

Type and topotype in the herbarium of Oregon State College, Corvallis, Oregon. Type (OSC *10614*) collected near Hermiston,

Umatilla Co., Oregon, on May 13, 1915, by H. S. Jackson. Topotype (OSC *10876*) collected at the same location by H. S. Jackson on May 12, 1915.

These two specimens, identified by H. S. Jackson as *Peronospora* sp., were loaned by L. F. Roth. Of the other species reported on members of the Scrophulariaceae, *Per. Linariae* Fuckel and *Per. Melampyri* (Bucholtz) Davis, both possess oogonia with thickened walls and hence belong in de Bary's Section Parasiticae (**2**). The oospores of *Per. sordida* Berk. & Br. have been described but once ("Dans le tissu bruni qui forme les taches les plus vieilles, on trouve un grand nombre d'oospores dont la mem-

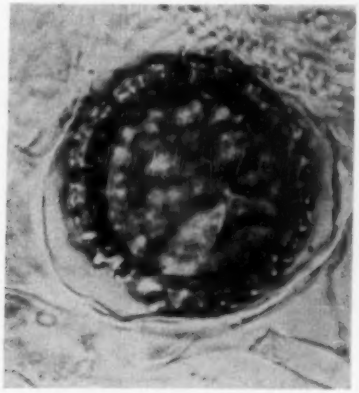


FIG. 1. Oospore of *Peronospora Jacksonii*. × 900.

brane, très épaisse et réfringente, et" (est?) "appliquée contre l'épispore qui formait les parois de l'oogone" [**8**, p. 284]). On the basis of this description *Per. sordida* is assigned to the Section Effusae, as are *Per. Antirrhini* Schroet. (**10**) and *Per. grisea* (Unger) Unger. Oospores of the latter two species are common, the exospore being irregularly thickened and wrinkled. Gäumann (**7**) refers the other species recognized by him for which oospores are known to the Section Effusae. *Per. Jacksonii* is easily distinguished from the preceding species on the basis of oospore characters. The exospore (FIGS. 1, 4B, 4C) is such that the species must be referred to Section Verrucosae (**6**).

Peronospora Nemophilae sp. nov.

Maculis incertis, saepe totum segmentum vel paene totum folium decolorantibus. Caespitulis sparsissimis, floccosis, albis; conidiophoris hypophyllis, non septatis, hyalinis, 1–3 coalitis e stomatibus exeuntibus, 250–725 μ altis, trunco ½ vel minus totius altitudinis efficiente et frequenter, praesertim longioribus conidiophoris, multo minus, 6–9 μ crasso, raro basi leviter tumida (13 μ), 5–7ies subdichotome ramosis, ramis primariis leviter curvatis, angulo acuto insertis; furcis terminalibus apice subacutis, inaequalibus, angulo acuto vel obtuso, at plerumque recto, insertis, rectis vel leviter curvatis, plerumque 5–12 μ longis, ramo uno ramum praecedentem continuente et recto vel incurvato; ramo altero breviore et recto vel leviter retroflexo. Conidiis hyalinis, subglobosis vel late ellipsoideis, apice obtusis, haud porosis, basi pedicellatis, 19–24 × 15.5–19.5 μ, hypha germinantibus; haustoriis vermiformibus;

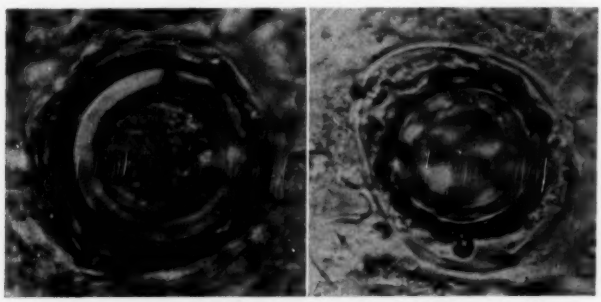


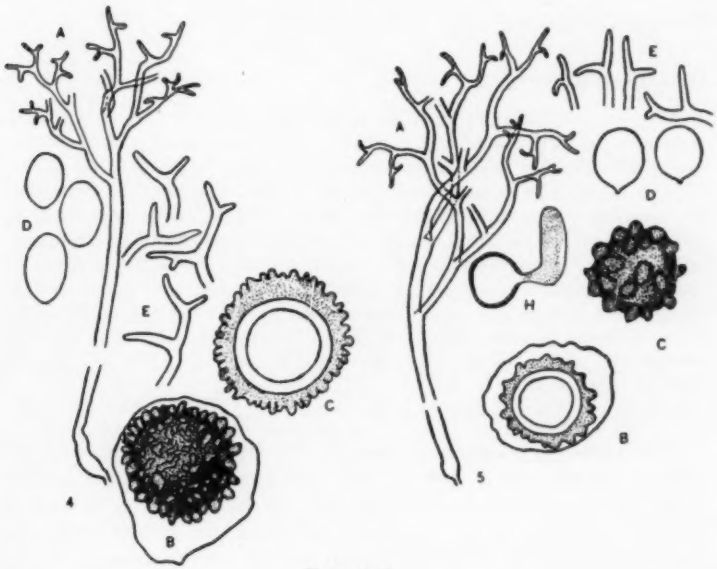
FIG. 2. Oospore of *Peronospora Hydrophylli* Waite. × 900.
FIG. 3. Oospore of *Peronospora Nemophilae*. × 900.

oogoniis subglobosis, 40–50 μ diam., membrana tenui, hyalina; oosporis globosis, 28–42 μ diam., laxis, exosporio crassitudinis comparate aequalis, 6–9 μ, denique fulvo et tuberculato; endosporio hyalino, 3.5–4.5 μ crasso. (FIGS. 3, 5.)

Hab. in foliis vivis *Nemophilae sepultae* Parish.

Foliicolous; infected area indefinite, usually starting at the tip of one of the leaf segments, enlarging to involve the whole segment or most of the leaf, at first chlorotic, then yellowish, and finally brownish and necrotic, very sparse, floccose, white mat below; conidiophores hypophyllous, aseptate, 1–3 emerging from a stoma, 5–7 times monopodially branched, variable in height, 250–725 × 6–9 μ, base occasionally enlarged to 13 μ, crown at most ½ the total height and usually much less, especially in the taller conidiophores; main branches somewhat curved, inserted at acute angles; ultimate branches subacute, apically unequal, inserted at acute to obtuse angles, but usually at approximately right angles, straight

or slightly curved, mostly 5–12 μ long, the axial the longer and straight to incurved, the abaxial straight or recurved; conidia hyaline, subspherical to broadly ellipsoid, basally pedicellate, 19–24 × 15.5–19.5 μ, germinating by a germ tube; haustoria vermiform; oogonia subspherical, 40–50 μ in diam., wall thin, hyaline; oospores 28–42 μ in diam., loose in the oogonia, exospore relatively uniform in thickness, 6–9 μ, at maturity dark brown and tuberculate, endospore 3.5–4.5 μ. (FIGS. 3, 5).



FIGS. 4–5.

On Hydrophyllaceae: oospores and conidia on *Nemophila sepulta* Parish.[3]

Type in the herbarium of The State College of Washington (WSC–PP *20470*). Collected near Gamm Creek Camp, Dose-

[3] A specimen of *Per. Nemophilae* has been found among the unidentified specimens in the Dudley Herbarium of Stanford University. The specimen bears the following data: "on *Nemophila parviflora*" Doug.; "by path in rear of Mrs. King's Home, King's Mt." San Mateo Co., California; "June 11, 1906; W. R. Dudley, Collector." This specimen contains both oospores and conidia and agrees with the type specimen cited above. Specimens from the Dudley Herbarium were loaned through the kindness of Mrs. Roxana Ferris, Curator.

wallips River, Jefferson Co., Washington, by C. G. Shaw and W. D. Yerkes, Jr., June 19, 1949.

The only downy mildew previously described on a member of the Hydrophyllaceae is *Per. Hydrophylli* Waite occurring on *Hydrophyllum* spp. and on *Phacelia* spp. The oospores of *Per. Hydrophylli* (FIG. 2) place it in the Section Effusae, while *Per. Nemophilae* (FIGS. 3, 5B) belongs in the Section Verrucosae (6). The oospores of *Per. Hydrophylli* are much larger, 38–54 μ in diameter, than those of *Per. Nemophilae*.

Plasmopara Cercidis sp. nov.

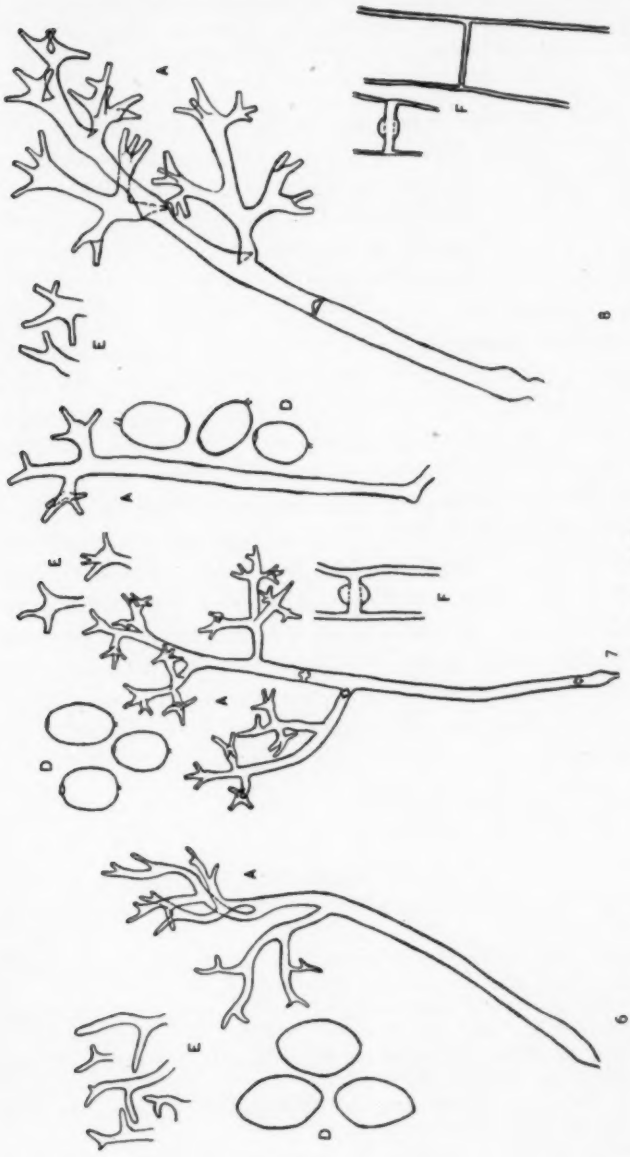
Maculis epiphyllis et hypophyllis, primo supra difficiliter visibilibus, dein brunneis; caespitulis floccosis, subluridis vel albo-sulfureis. Conidiophoris hypophyllis, hyalinis, septatis, 220–460 μ altis, trunco ⅓ vel ½ totius altitudinis efficiente, 6–9 μ crasso, plerumque basi leviter tumida (11 μ), fasciculatis, nonnullis e stomatibus exeuntibus, 3–4ies pinnatis; ramis primariis 4–5, rectis vel ramis humilioribus leviter sursum curvatis, angulo recto vel humilioribus angulo acuto insertis, ramis subultimis in 2–5 ramulos divaricatos exeuntibus; ramulis ultimis rectis vel leviter tortuosis, conicis, 4–8(–9.5) μ longis, apice truncatis. Conidiis hyalinis, ellipsoideis vel late ovoideis, apice poris, inconspicuis praeditis, basi inconspicue pedicellatis, 14.5–23 × 12–15.5 μ (longitudine media 18.8μ, latitudine media 13.8 μ). Oogoniis et oosporis ignotis. (FIG. 7.)

Hab. in foliis *Cercidis canadensis* L.

Foliicolous; infected areas angular, limited by the veins, younger lesions inconspicuous from above and appearing light cinereous from below, old lesions red-brown to dark brown, necrotic, and covered with the thin, cream-colored mat below; conidiophores hypophyllous, with plug-like septations, hyaline, fasciculate, up to six or more emerging from a stoma, 3–4 times monopodially branched, 220–460 × 6–9 μ, often bulbously enlarged basally up to 11 μ; crown ⅓ to ½ the total height, the crown being a greater portion of the total height in the shorter conidiophores, primary branches 4–5 in number, straight or the lower-most occasionally arching upwards, arising at right angles or the lower-most acutely inserted; ultimate branches 2–5 in number, conic, straight or slightly tortuous, apically truncate, 4–8 μ long, a few up to 9.5 μ; conidia hyaline, ellipsoid to broad ovate, 14.5–23 × 12–15.5 μ (Mean 18.8 × 13.8 μ), with a short, indistinct basal pedicel, and an indistinct apical pore; oogonia and oospores unknown. (FIG 7.)

On Leguminosae: conidia on *Cercis canadensis* L.

Type and topotype in the Division of Mycology and Disease Survey Herbarium, U. S. Bureau of Plant Industry, Soils, and



FIGS. 6–8.

Agricultural Engineering, Beltsville, Maryland. Type collected at Signal Mt., Chattanooga, Hamilton Co., Tennessee, Aug. 20, 1927, by Mrs. R. H. Williams. Topotype collected at the same location on Sept. 9, 1927, by W. J. Forbes.

A single reference to this species exists in the literature (1). The type specimen, identified as *Plasmopara* sp., is included in a list of "Some Recent Specimens" received at the Office of Mycology and Disease Survey with the comment that this is "apparently a new downy mildew for this country" (1). The specimen was transmitted by C. D. Sherbakoff.

J. L. Stevenson lent the two collections for study. Conidia and conidiophores are present in both specimens, although scanty in the topotype. Oospores were not observed in either of the two specimens. Attempts to locate additional material were unsuccessful. L. R. Hesler, in personal correspondence, informed the writer that he has no record of additional collections in Tennessee.

Both the conidiophores and the conidia have the characters of the genus *Plasmopara*. The conidia are both poroid ("papillate") and pedicellate. The primary branches of the conidiophores arise at right angles, pluglike septations are present in the trunk of the conidiophores, and the truncate ultimate branches occur in groups of 2–5. No species of the Peronosporaceae has previously been described on the genus *Cercis;* nor is the author aware of any species of *Plasmopara* previously reported on a member of the Leguminosae.

STATE COLLEGE OF WASHINGTON,
PULLMAN, WASHINGTON

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EXPLANATION OF FIGURES

Photographs in figures 1–3 taken by W. D. Yerkes, Jr.

Figures 4–8 drawn with the aid of a camera lucida. A, Conidiophore × 275. B, Oogonium with enclosed oospore × 325. C, Oospore × 325. The exospore has been stippled in all oospore drawings as an aid to distinguishing the various structures. D, Conidia × 325. E, Ultimate branches × 325. F, Septation × 900. H, Germinated conidium × 325.

FIG. 4. *Peronospora Jacksonii.* FIG. 5. *Peronospora Nemophilae.* Fig. 6. *Peronospora Davisii.* FIG. 7. *Plasmopara Cercidis.* FIG. 8. *Plasmopara Myosotidis.*

THE RELATIONSHIP OF PUCCINIA PRAEGRACILIS AND P. CONNERSII [1]

D. B. O. Savile

The writer is indebted to Dr. Ivar Jørstad for pointing out (*in litt.*) the probable identity of *Puccinia connersii* Savile, on *Deschampsia atropurpurea*, with *P. praegracilis* Arth., on *Agrostis thurberiana*.

Puccinia praegracilis was described by Arthur (**1**) from specimens collected by E. W. D. Holway at Glacier, in the Selkirk Mts., B. C., where he had already found *Aecidium graebnerianum* on *Habenaria gracilis* in 1901. In a note accompanying the type collection, Holway stated that the grass rust "grew adjacent to the *Habenaria* aecidium, and nowhere else." He had two stations for the aecial stage but could not revisit the larger one because continuous fog and rain obscured his landmarks. The second colony consisted of half a dozen plants, between which the rusted *Agrostis* occurred. In his original description Arthur quoted this exclusive association, which was repeated in a further collection from a different station at Glacier in 1907, but he ignored it later (**2, 3**) when he submerged *Puccinia praegracilis* in *P. coronata*. This treatment, together with its occurrence upon *Agrostis*, caused the writer (**4**) to overlook *P. praegracilis* when seeking affinities of *P. connersii*.

Through the courtesy of Dr. G. B. Cummins, both of Holway's collections on *Agrostis thurberiana* have been examined. In the type specimen, taken on 5 Sept. 1902, telia had almost completely superseded uredinia, clearly indicating that this rust does not overwinter in the uredinial stage. This fact is also plain from the distribution pattern of the sori in both collections; for they are not crowded on the basal leaves, but are scattered up and down the plants and are slightly heavier on upper than on basal leaves. In

[1] Contribution No. 1060 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

alpine, as in subarctic, situations meager uredinial spread makes the association between aecia and telia very close. Consequently, the distinction between uredinial and aecial origin of grass and sedge rusts is usually clear to a trained observer even late in the season. There is no valid reason to doubt that Holway's rust on *Agrostis* stemmed from aecia on *Habenaria*, just as did the writer's rust on *Deschampsia*.

Examination of Holway's collections has allowed some expansion of the description of *Puccinia praegracilis*, as follows: urediniospores 16–21 × (13.5)–16–19 μ; wall 1.2–1.6 μ, subhyaline to pale greenish yellow, finely echinulate at 1.5–2.5 μ spacing; pores several, scattered, visible only in the spores with the thickest walls. Teliospores 29–47 × 11–17 μ; wall 0.5–1 μ below, 2.5–4 μ at apex excluding appendages, yellow-brown to chestnut; usually a few digitate appendages 2–5 μ long. A single, seemingly aberrant, teliospore was 51 μ long including appendages 9 μ long.

The difference between this range of measurements and the overall dimensions of *Puccinia coronata*, as summarized by the writer (**4**), is slight; but it must be remembered that the latter embraces several entities that differ appreciably in morphology as well as in host relationship, some of which might well be treated as individual species. No single one of these entities agrees exactly with *P. praegracilis*. The somewhat short teliospores, small urediniospores and thin urediniospore walls, taken with the occurrence of aecia on a monocotyledonous plant, make it desirable to recognize *P. praegracilis* as a distinct species.

Comparison of *Puccinia praegracilis* and *P. connersii* shows them to be too similar in all respects to be maintained as separate species. However, *P. connersii* has somewhat thinner urediniospore walls (0.5–1.2 μ) and slightly shorter and broader teliospores (mostly 25–36 × 12–20 μ) than *P. praegracilis*, differences that indicate host specialization, at least upon the grass hosts. It is accordingly proposed as a variety of *P. praegracilis*.

Puccinia praegracilis Arth. var. **praegracilis** *var. nov.*

I on *Habenaria gracilis* (*Aecidium graebnerianum* P. Henn. *p.p.*); II, III on *Agrostis thurberiana*, Glacier, B. C.

Puccinia praegracilis Arth. var. **connersii** (Savile) *comb. nov.*

(Syn. *P. connersii* Savile). I on *Habenaria dilatata* (*Aecidium graebnerianum* P. Henn. *p.p.*); II, III on *Deschampsia atropurpurea*, Great Whale River, Que.

As Dr. Jørstad has pointed out, *Deschampsia* (*Vahlodea*) *atropurpurea* is not a typical species of *Deschampsia*. The rust on it may, therefore, be unable to attack other species of this genus. In view of the realization that aecia on *Habenaria* may go to more than one grass genus, the possibility of the occurrence of further races or varieties on yet other grasses must now be considered. Consequently the telial host associated with Mr. Payette's collection on *H. psychodes* at Rivière Ouelle, in eastern Quebec, previously suggested by the writer to be *Deschampsia caespitosa* var. *intercotidialis*, remains in doubt.

Since the above report was prepared the writer has received from Mr. F. Roll-Hansen a specimen of *Aecidium graebnerianum* from the southwest part of Cape Breton Island, N. S. When he found it on 29 June 1950, the rusted plants had not started to flower. From the leaf form the host could equally well be *Habenaria lacera, H. fimbriata* or *H. psychodes;* but, from its bog habitat, it is probably *H. lacera.* The rust infection is very heavy, suggesting that the grass host was also a bog species.

DIVISION OF BOTANY AND PLANT PATHOLOGY,
SCIENCE SERVICE, DEPARTMENT OF AGRICULTURE,
OTTAWA, CANADA

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NOTES AND BRIEF ARTICLES

The Status of the Generic Names Mycoacia and Oxydontia

As is the case in other groups of the Homobasidiomycetes, the generic limitations of forms bearing the hymenium on downward-directed teeth and grouped as the family Hydnaceae, itself a taxon with rather vaguely defined boundaries, are still in an unsatisfactory state. This note is concerned with certain members of the group which have been assigned to *Hydnum* (in the wide sense), *Acia*, *Mycoacia* and *Oxydontia*.

In 1879, Karsten (Medd. Soc. Faun. Fl. Fenn. **5**: 42) established the genus *Acia* as a segregate for certain resupinate members of the Hydnaceae, describing it in the following terms: "Receptaculum resupinatum, tenerrimum aut fere nullum." The emphasis was on the resupinate habit and thin receptacle. Karsten did not designate a type, but listed 31 species, the first of which was *Acia fuscoatra* (Fr.) Karst. based on *Hydnum fusco-atrum* Fr. Two years later Karsten (Medd. Soc. Faun. Fl. Fenn. **6**: 15–17. 1881) transferred seven of these species to other genera of the Hydnaceae.

In 1900, Patouillard (Tax. Hymen. 68) adopted Karsten's genus but emphasized the tendency of the spines to be clustered at the bases, approaching the polypores. While not so stated, this was, in effect, an emendation of Karsten's genus. Patouillard cited 7 species, including *Acia fuscoatra* (Fr.) Karst. Some of the other species would now probably be assigned to different genera.

When the original author of a genus designates no holotype at the time of publication, a lectotype may be selected by a later author from the species originally cited. In 1902, Banker (Bull. Torrey Club **29**: 444) proposed *Hydnum fusco-atrum* Fr. as the type of Karsten's genus and since this was one of Karsten's original species the selection is binding.

In 1928, Killermann (E. & P. Nat. Pfl. ed. 2. **6**: 159–160) reduced *Acia* Karst. to a section of *Odontia*, retaining the type species in the section.

Bourdot and Galzin (Hymén. France 414. 1927), Rea (Brit. Basid. 31. 641. 1928), and other European writers, following Patouillard, restricted *Acia* to adnate, ceraceous forms only. The species listed under *Acia* Karst. seemingly for the most part belong to other genera. *Acia* Karst. 1879 as a genus of the Hydnaceae is invalid since it is a homonym of *Acia* Schreb. 1791, applied to a genus of the Rosaceae. For this reason, Donk (Med. Ned. Mycol. Ver. **18–20**: 150. 1931) proposed the genus *Mycoacia* as a substitute for the invalid name *Acia* in Patouillard's sense, citing "*Acia* Pat." (there is no "*Acia* Pat."; Patouillard merely adopted and emended Karsten's genus) and *Acia* Karst. *pro parte* as synonyms. He included four species: *M. fuscoatra* (Fr.) Donk (= *Hydnum fusco-atrum* Fr.), *M. setosa* (Pers.) Donk, *M. stenodon* (Pers.) Donk, and *M. uda* (Fr.) Donk. Of these, the first is indicated as the type. The essential characters of the type of the genus, as stressed by Donk, were the effused, waxy, strongly adnate basidiocarp and the slender, subulate, 2–6 mm. long teeth.

This, it appears, is contrary to Fries's own description of *Hydnum fusco-atrum* Fr., part of which reads as follows: "Aculei minus conferti, subbreves, obtusiusculi juniores cervini" (Syst. Myc. **1**: 416. 1821). The emphasis is on the less crowded, rather short, blunt-tipped teeth, branched or fimbriate when young.

Mycoacia fuscoatra (Fr.) Donk, and *M. uda* (Fr.) Donk, have been included in the genus *Odontia* by different authors, including Miller (Mycologia **26**: 30–32. 1934), Miller and Boyle (Univ. Iowa Stud. Nat. Hist. **18**[2]: 34–36. 1943) in their recent revision of the Iowa species, and Brown (Bot. Gaz. **96**: 648–653. 1935). It was demonstrated by Brown that *Odontia fusco-atra* (Fr.) Bres. is a true *Odontia* and produces cystidia in culture.

On the basis of the fairly ample material at my disposal in the mycological herbarium of the State University of Iowa, I am convinced that Miller and Boyle were justified in retaining both *Mycoacia fuscoatra* (Fr.) Donk, and *M. uda* (Fr.) Donk in the genus *Odontia*.

In 1933, Miller (Mycologia **25**: 294. 1933) established the genus *Oxydontia*, unaware that Donk had already proposed his genus *Mycoacia*. Miller established *Oxydontia* to replace *Hydnum* as used by Quélet for resupinate forms and *Acia* Karst., neither name being ten-

able. *Hydnum* as used by Quélet (in Cooke & Quélet, Clav. Hymen. Europ. 200. 1878) is invalid, since the segregate was preoccupied by *Hydnum* L. emend. S. F. Gray (Nat. Arr. Brit. Pl. **1**: 650. 1821), who retained the name *Hydnum* for certain of the fleshy, mesopodous species. *Oxydontia*, as defined, referred to species characterized by a resupinate effused fructification, adnate or separable, floccose, fleshy or ceraceous, with typical long-subulate spines, and lacking cystidia. *Oxydontia setosa* (Pers.) Miller (= *Hydnum setosum* Pers.) is designated as the type and 5 additional species are included. Donk (Med. Ned. Mycol. Ver. **18–20**: 152–153. 1931) had already transferred *Hydnum setosum* Pers. to *Mycoacia*.

In 1943, Miller and Boyle (Univ. Iowa Stud. Nat. Hist. **18**[2]: 38) adopted Donk's genus *Mycoacia* for what Miller had called *Oxydontia*, but cited *Hydnum setosum* Pers. incorrectly as the type. The type of *Mycoacia* must remain *Hydnum fuscoatrum* Fr. However, Miller and Boyle, as already pointed out, retained that species in *Odontia* as *O. fusco-atra* (Fr.) Bres.

The distinction between *Mycoacia* and *Odontia* according to Donk (Med. Ned. Mycol. Ver. **18–20**: 100. 1931) is based on the waxy texture, adherent to the substratum, of the former, as opposed to the membranaceous or floccose consistency of the latter; long teeth, 1–10 mm., with entire apices (sometimes more or less branched) as opposed to shorter, irregular teeth; and the absence of cystidia and gloeocystidia (but mostly with cystidioles) as opposed to the presence of cystidia or gloeocystidia.

Such a separation is artificial. Many of the species recognized in *Odontia* are described as waxy and very adherent. The entire tip of the spine is very variable in the same fructification. It is suggested that the name *Mycoacia* be rejected, since it is likely to lead to confusion with *Odontia*.

Miller made the basic distinction between *Odontia* and *Oxydontia* the presence or absence of cystidia. Although this distinction also is somewhat artificial, it has the advantage of being useful and workable.

To summarize: (1) Karsten published the genus *Acia* with 31 species, without typifying it. The name is untenable and the genus, as proposed, was heterogeneous, as was recognized by

Karsten himself in his later writings. (2) Banker suggested *Hydnum fusco-atrum* Fr. as a lectotype. (3) Donk's genus *Mycoacia* is not the exact equivalent of Miller's *Oxydontia* since Miller made the absence of cystidia part of his generic diagnosis. (4) Both genera include resupinate species with soft or subwaxy consistency, long, slender and typically terete spines and white spores. (5) *M. uda* (Fr.) Donk and *M. fuscoatra* (Fr.) Donk are referred to *Odontia* by Miller and other authors. (6) It is recommended that the genus *Oxydontia* Miller be maintained. It includes a greater range of species than *Mycoacia* Donk and the absence of cystidia is a more workable generic character than the texture of the fructification.—M. A. RAGAB.

NEW EVIDENCE FOR TYPIFICATION OF AURICULARIA

G. W. Martin (Amer. Mid. Nat. **30**: 77–82. 1943) and M. A. Donk (Bull. Bot. Gard. Buitenz. III. **18**: 83–167. 1949) have discussed the typification of *Auricularia*, giving their interpretation of what should be considered the earliest legitimate starting point for the genus. Martin concluded that "*Auricularia*, in the modern sense, must be attributed to Persoon," hence, proposed the adoption of *Auricularia* Persoon Myc. Eur. **1**: 97. 1822, with *Thelephora mesenterica* Bolt. as the type species.

It was Donk's opinion that the work of Brongniart (Dict. Sci. Nat. **33**: 577. 1824), based on *Auricularia* Bulliard should serve as the starting point and be adopted as a nomen conservandum.

It was the opinion of both authors that a search of the literature of the period between 1821–1825 might reveal new evidence which would result in a need for further revision of their proposals.

Such evidence has recently been discovered by Dr. D. P. Rogers in the form of a work by F. V. Mérat entitled "Nouvelle Flore des Environs de Paris," ed. 2. 1821. Since 1821 is the earliest legitimate starting point for fungi of this group, it is suggested that *Auricularia* Bull. ex Mérat, Nouv. Fl. Env. Paris, ed. 2, **1**: 33. 1821, be adopted, with *A. tremelloides* Bull. as the type species.

It should be noted that *A. tremelloides* Bull. = *Thelephora mesenterica* (Dicks.) Pers. 1822. According to the International

Rules as amended at Stockholm in 1950, Article 20(f) now states: "Fungi caeteri published in other works between the dates of the first and last parts of the Systema which are synonyms or homonyms of names of any of the *Fungi caeteri* included in the Systema do not affect the nomenclatural status of names used by Fries in this work." Fries in Elenchus Fungorum **1**: 154 refers to *Phlebia mesenterica*, so that the specific epithet is to be retained as *mesenterica*. Persoon's notation "mala," in Myc. Eur. **1**: 97, as a commentary on Buillard's plate 290 is certainly justifiable; nevertheless, there can be no doubt that *A. tremelloides* Bull. is the fungus to which the name *A. mesenterica* is now commonly applied. Among others who have concurred in this opinion were: Duby in Bot. Gall. **1**: 773, who referred to it as *Phlebia mesenterica;* Berk. in Hook., Smith's Eng. Fl.-Crypt. **2**: 162 (as *Phlebia mesenterica*); Bourd. & Galz. Hym. Fr. 15 (as *A. mesenterica*).—B. LOWY.

CORRECTION—RE DERMEA

Due to a personal error the accounts of the genus *Dermea* by Fries in the Elenchus Fungorum and the Summa Vegetabilium Scandinaviae were mixed up in my notes. Unfortunately the original books are not available locally. Fries did not adopt the spelling *Dermatea* until the later work and, therefore, the interpretation given by myself in Mycologia **43**, p. 114, is wrong and the spelling *Dermea* should stand.—J. WALTON GROVES.

REVIEWS

THE NORTH AMERICAN CUP-FUNGI (INOPERCULATES), by Fred Jay Seaver. 428 p., 150 pl. 1951. New York, publ. by author.

This companion volume to the author's North American Cup-fungi (Operculates) published in 1928 with a supplement in 1942 comprises three families, Geoglossaceae, Helotiaceae, and Cenangiaceae of the Pezizales. The species of Helotiaceae are divided into five tribes: Sclerotineae, Helotieae, Mollisieae, Ascotremelleae, and Lachnelleae. Keys are given to tribes, genera, and species. Each is described and synonyms, distributional records, illustrations, and exsiccati are listed. Some of the illustrations, which combine photographs and drawings of microscopical details, are reproduced from previous papers in MYCOLOGIA, while many are new. Citations to the publications of generic and specific names are given but there is no formal bibliography. As pointed out by the author, the nomenclature used does not always conform to the International Rules. As is inevitable in dealing with so large a group of fungi, in regard to which widely diverse views are held, the taxonomic treatment may be expected to meet with some objections. Outstanding monographs on certain groups and genera have been followed to a large extent, with modifications and additions, such as Durand in the Geoglossaceae, Whetzel in the Sclerotineae, White in *Helotium*, and Groves in *Pezicula* and *Dermea*. White's conception of *Rutstroemia* is, however, not recognized and some significant papers, such as those of Buchwald and Dennis, are too recent to have been given consideration. Many well known and established genera are relegated to synonymy, for instance *Ascocalyx*, *Diplocarpon*, *Drepanopeziza*, *Hyaloscypha*, *Cistella*, and *Unguicularia*. In some cases spore septation is apparently used as the chief basis for generic distinction (*Cenangella*, *Dermatella*), while in others (*Godronia*) species are included in which spores range from unicellular to many-septate. *Lachnella* Fries for the controversial *Dasyscypha-Lachnum* complex does not seem too happy a

choice of generic name. Although this genus is typified by *Peziza alboviolascens* and therefore generally regarded as a synonym of the basidiomycete genus *Cyphella*, the author advocates that it be conserved for a genus of the Discomycetes. *Helotiella* and *Godronia*, which are both names of uncertain application and doubtful validity, are accepted by Seaver. Occasional misprints, omissions and errors in citation are regrettable, but do not seriously detract from the book's value as a comprehensive summary of the North American inoperculate Discomycetes.—EDITH K. CASH.

MYCOTROPHY IN PLANTS. Lectures on the Biology of Mycorrhizae and related Structures, by Arthur P. Kelley. VOL. 22 of "A New Series of Plant Science Books," edited by Frans Verdoorn. i–xvi + 223 pp., 16 figs., 5 plates. The Chronica Botanica Co., Waltham, Mass. 1950. Price $4.50.

In view of the increasing importance of the study of mycotrophy, there has been need for a comprehensive survey which will assemble the scattered information on the subject and present it in suitable form for mycologists, teachers of botany and students. This need is effectively met by Kelley's volume. The twelve "lectures" discuss the history of the subject, the occurrence of mycorrhizae, the fungi involved, fossil evidence, distribution, relation to environment, mycothalli and mycorrhizomes, mycodomatia, the structure of mycorrhizae, obligate symbiosis, theories of mycotrophy, mycotrophic phagocytosis. The bibliography of 24 pages lists over 500 references and additional papers are cited in the introduction.

The treatment throughout is factual and condensed, probably a necessary consequence of the attempt to give in concise form the substance of so many contributions. In a number of instances, contradictory evidence is pointed out and in this and in other ways suggestions for profitable lines of possible investigation are given. It may be expected that the book will be extremely useful wherever fungi or the green plants associated with them are seriously studied. —G. W. M.

MANUSCRIPT

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